

Research DAY

Department of Medicine 2012

GRADUATE STUDENTS | RESIDENTS | POSTDOCTORAL FELLOWS

THURSDAY, MAY 17, 2012

ORAL PRESENTATIONS

CLASSROOM D

2F1.04 WALTER MACKENZIE CENTRE

POSTER PRESENTATIONS

LOWER LEVEL

JOHN W SCOTT HEALTH SCIENCES LIBRARY



UNIVERSITY OF ALBERTA
FACULTY OF MEDICINE & DENTISTRY
Department of Medicine

Chair's Welcome

Barbara J. Ballermann, MD

“Welcome to our Research Day - one of the most important and rewarding days in our academic year! It is a day when we hear about the exciting research projects in which our Graduate Students, Post Doctoral Fellows, Core Internal Medicine and Subspecialty Residents are involved. This year we are fortunate to have as our guest oral adjudicator, Dr. Gary Curhan, Professor of Medicine and Epidemiology, Harvard School of Medicine.

Research Day gives the opportunity for all Department members and guests to interact with our young researchers. We currently have a total of 60 Graduate Students, 34 Postdoctoral Fellows and 175 Core Internal Medicine and Subspecialty Residents. As such, I would encourage you to attend the oral presentations in Classroom D and visit at least three posters which will be located in the lower level of the John W Scott Library.

Enjoy today and be sure to join us for the presentation of awards at the conclusion of the afternoon oral presentations.”



EVANGELOS MICHELAKIS, MD, ASSOCIATE CHAIR, RESEARCH

“During the second Persian invasion to Greece (September of 480 BC) a critical battle took place in Thermopylae (“Hot Gates”) a narrow coastal passage in central Greece. The Greek army, under the Spartan King Leonidas, of about 7000 men was called to fight the Persian army, under King Xerxes, of more than 500,000. After the second day of battle, a local resident named Ephialtes betrayed the Greeks by revealing a small path that led behind the Greek lines. Aware that his force was being outflanked, Leonidas dismissed the bulk of the Greek army, and remained to guard the rear with 300 Spartans. They all died and the Persians (Medes) went through. The delay however was critical in organizing the Greek navy that shortly after won a decisive victory against the much stronger Persian navy (in the battle of Salamina) resulting in the Persian withdrawal from the invasion to Europe.”

....Such should be your commitment to pursuing your ideas in your careers that are just starting and such your courage and compassion in defending what you believe is right. Guarding your own Thermopylae is a more important and admirable goal than money, grants, papers, awards.....

THERMOMYLAE

*Honor to those who in the life they lead define and guard a Thermopylae.
Never betraying what is right,
consistent and just in all they do
but showing pity also, and compassion;
generous when they are rich, and when they are poor, still generous in small ways,
still helping as much as they can; always speaking the truth, yet without hating
those who lie.*

*And even more honor is due to them
when they foresee (as many do foresee)
that in the end Ephialtis will make his appearance, that the Medes will break
through after all.*

CP Cavafy (~1913)

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**Key: GS = Graduate Student, PDF = Post Doctoral Fellow
SR = Subspecialty Resident, CR = Core Internal Medicine Resident**

Research Day Guest Adjudicator

Gary Curhan, MD, ScD

Dr. Curhan is currently Professor of Medicine and Epidemiology at Harvard Medical School and Harvard School of Public Health.

He earned his medical degree from Harvard Medical School in Boston in 1985. His residency training in Internal Medicine and fellowship in Nephrology was done at the Massachusetts General Hospital and Harvard Medical School before completing his Master of Science Degree in 1991 and Doctor of Science Degree in 1996 both at Harvard School of Public Health.



Active research interests include the epidemiology of nephrolithiasis, risk factors for renal function decline, epidemiology of hearing loss, cardiovascular disease, health effects of analgesic use, novel risk factors for hypertension, epidemiology of gout, and incontinence. Additional areas of active research include epidemiological studies of primary hyperparathyroidism, acute renal failure, pneumonia, and shingles. Risk factors being explored include dietary intake, 24-hour urine composition, urine and blood biomarkers, and genetic factors. Dr. Curhan spends approximately 80% of his time on these research activities and the remainder of his time on mentoring, teaching and clinical and administrative responsibilities. He is the Editor-in-Chief for the Clinical Journal of the American Society of Nephrology (CJASN). He serves on national committees including the Advisory Council for the National Center for Complementary and Alternative Medicine at NIH.

Research Day Guest Adjudicator

Lori J. West, MD, DPhil

Dr. West is Professor of Pediatrics, Surgery and Immunology at the University of Alberta and the Director of Research at the Alberta Institute of Transplantation Sciences, a Senior Scholar for Alberta Innovates – Health Solutions (AIHS) and a Tier 1 Canada Research Chair in Cardiac Transplantation. She is a world leader in pediatric cardiac transplantation and transplant immunobiology, including crucial translation of basic concepts and findings from murine models to clinical application in pediatric heart transplantation. Her clinical work resulted in a pioneering strategy for increasing donor availability for infants by crossing the ABO barrier, which has had a major global impact on infant heart transplantation. Her investigations of the immune development of infants after ABO-incompatible transplantation led to the first demonstration of neonatal transplantation tolerance in humans. She is now leading an international multi-site collaboration in the study of this unique patient population globally. The overall umbrella of Dr. West's cardiac transplant research program includes much-needed attention to drug therapy strategies in pediatric transplant populations and outcomes analyses, including quality-of-life outcomes of pediatric organ transplantation. Dr. West is also a leader in basic science research in transplantation. The work of her team in murine models of neonatal tolerance has demonstrated that neonatally-induced acceptance of cardiac allografts is related to induction of regulatory T cells by the cardiac graft, a finding with potentially important clinical applications for antenatally diagnosed patients with congenital heart disease requiring neonatal heart transplantation. Her research team has also developed a murine model of ABO-incompatible transplantation using a novel lentiviral gene transfer strategy, which will provide a new platform for investigation of research questions in antibody-mediated rejection and B cell tolerance. Dr. West is the immediate past-president of the International Society of Heart and Lung Transplantation.



Panel of Judges

Gary Curhan, MD, ScD

Professor of Medicine and Epidemiology
Harvard Medical School

Lori West, MD

Professor of Pediatrics
Surgery and Immunology
University of Alberta

Barbara J. Ballermann, MD

Professor of Medicine
Chair, Department of Medicine
University of Alberta

Morning Session Chair

Dr. Ross Tsuyuki

Coordinator, Graduate Education Program

Afternoon Session Chair

Dr. Darryl Rolfson

Director, Postgraduate Medical Education

Meeting at a Glance

7:55-8:15	Welcome Address
8:15-9:30	Oral Presentations (GS)
9:30-9:45	Break
9:45-11:00	Oral Presentations (PDF)
11:00-12:50	Poster Presentations and Lunch
1:00-1:15	Awarding the Department of Medicine Translational Research Fellowship
1:15-2:45	Oral Presentations (SR)
2:45-3:00	Break
3:00-4:00	Oral Presentations (CR)
4:15	Award Winners

Morning Session

Oral Presentations

Graduate Students & Post Doctoral Fellows

8:15 – 11:00 a.m.

Classroom D, 2F1.04 WMC

	Name		Title	Page
7:55	Welcome Address			
8:15	Gill Richdeep Supervisor: Raj Padwal	GS	Prioritization and Willingness-to-Pay for Bariatric Surgery: the Patient Perspective	22
8:30	Haromy, Alois Supervisor: Dr. Evangelos Michelakis	GS	Mitochondrial Activation Suppresses Hypoxia-Inducible Factor 1 α Signaling in Cancer	23
8:45	Bredo Graeme Supervisor: Lisa Cameron	GS	Influence of IL-25 on Th2 Lymphocytes and Allergic Inflammation	24
9:00	Maulik Mahua Supervisor: Satyabrata Kar	GS	Influence of Cholesterol Accumulation in a Mouse Model of Alzheimer's Diseases	25
9:15	Mojiri Anahita Supervisor: Nadia Jahroudi	GS	Hypoxia exposure results in upregulation of VWF gene and its de novo activation in lung microvascular endothelial cells	26
9:30	Break			

Morning Session

Oral Presentations

Graduate Students & Post Doctoral Fellows

8:15 – 11:00 a.m.

Classroom D, 2F1.04 WMC

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9:45	Reinke Stacey Supervisor: Christopher Power	PDF 1H-NMR analyses of brain tissue and cerebrospinal fluid reveal distinct metabolomic profiles: a new strategy to diagnosing and understanding multiple sclerosis.	27
10:00	Ohland Christina Supervisor: Karen Madsen	PDF Enteropathogenic Escherichia coli (EPEC)-induced epithelial hyposecretion is associated with decreased membrane localization of CFTR	30
10:15	Walsh John Supervisor: Christopher Power	PDF Inflammasome Expression within the Brain	31
10:30	Wang Weiwei Supervisor: Andrew Mason	PDF Isolation Of The Human Betaretrovirus And Demonstration Of Integration Sites In Patients With Primary Biliary Cirrhosis	32
10:45	Haile Yohannes Supervisor: Fabrizio Giuliani	PDF The role of serpin3n as a potentially novel inhibitor molecule in granzyme B-mediated neuronal injury	33

11:00 Poster Sessions

Afternoon Session

Oral Presentations

**Subspecialty Residents & Core Internal Medicine
Residents
1:00 – 3:30 p.m.
Classroom D, 2F1.04 WMC**

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1:15	Kaila Ken Supervisor: Kevin Bainey	SR Percutaneous Coronary Intervention (PCI) Outcomes of South Asians with Acute Coronary Syndromes 34
1:30	D'Souza Pernilla Supervisor: Christopher Teshima	SR Early Experience Using A New Core Eus Biopsy Needle For The Diagnosis Of Solid Mass Lesions 35
1:45	Hoang Holly Supervisor: Wendy Sligl	SR Macrolide Use in the Treatment of Critically Ill Patients with Pneumonia: Incidence, Correlates, Timing and Outcomes 36
2:00	Bolster Lauren Supervisor: Nancy Zhu	SR Retrospective chart review of patients receiving Azacitidine (Vidaza®) in the Edmonton region of Alberta Health Services 37
2:15	Bello Aminu Supervisor: Marcello Tonelli	SR A Population-based study on care and clinical outcomes in remote-dwellers with heavy proteinuria 38

2:30 Break

Afternoon Session

Oral Presentations

**Subspecialty Residents & Core Internal Medicine
Residents
1:00 – 4:00 p.m.
Classroom D, 2F1.04 WMC**

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2:45	Albalawi Zaina Supervisor: Finlay McAlister	CR	How common are false positive and false negative findings in cardiovascular meta-analyses?	39
3:00	Sohaib Javaria Supervisor: Godfrey Man	CR	Use Of Hypnotics In Hospitalized Chronic Obstructive Pulmonary Disease Patients	40
3:15	Gurtu Vikram Supervisor: Evangelos Michelakis	CR	Initial Brain Natriuretic Peptide levels, but not hemodynamics, predict 5-year survival in patients with Pulmonary Arterial Hypertension	41
3:30	Al-Zahmi Fatmah Supervisor: Omar El-Agnaf	CR	α -synuclein levels in the blood as a potential biomarker for presymptomatic PD	43
3:45	Kahlon Sharry Supervisor: Summit Majumdar	CR	Obesity and Outcomes in Patients Hospitalized with Pneumonia	44
4:00	Conclusion of Oral Presentations			
4:15	Presentation of Awards to Winners			

Poster Presentations

**Core Internal Medicine & Subspecialty Residents,
Graduate Students & Post Doctoral Fellows**

All Day Viewing

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2	Das, Subhash Supervisor: Dr. Gavin Oudit	GS	Patho-physiology of Iron-Overload Cardiomyopathy: Linking Oxidative Stress to Diastolic Dysfunction- improvement with Resveratrol	47
3	Pedrycz, Barbara Supervisor: Dr. Thomas Mueller	GS	Oncostatin M Receptor deficiency is associated with less mortality and attenuated multi-organ dysfunction in sepsis	48
4	O'Shea, Daire Supervisor: Dr. Atul Humar	GS	Preventing HCV re-infection Post-transplant: In Vitro Evaluation of Novel agents providing Cross Genotype Protection against HCV	49
5	Kish, Lisa Supervisor: Dr. Karen Madsen	GS	Airborne particulate matter alters intestinal permeability and immune function through direct effects on innate immune cells	51
6	Al-Momany, Abass Supervisor: Dr. Barbara Ballermann	GS	CLIC5A Maintains Ezrin-Dependent Podocyte Structure and Function	53
7	Oster, Richard Supervisor: Dr. Ellen Toth	GS	Predictors of A1c among women with a history of gestational diabetes in Aboriginal communities of Alberta	54
8	Kolahdooz, Fariba Supervisor: Dr. Sangita Sharma	GS	Dietary adequacy of vitamin D and calcium among Inuit and Inuvialuit women of child-bearing age in Arctic Canada: A growing concern	55
9	Wang, Yanlin Supervisor: Dr. Satyabrata Kar	GS	Potential role of IGF-II receptor in APP processing	56

Poster Presentations

Core Internal Medicine & Subspecialty Residents, Graduate Students & Post Doctoral Fellows

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11	Baluch, Aliyah Supervisor: Dr. Deepali Kumar	GS	A Randomized Trial of High-dose Intradermal versus Intramuscular Seasonal Influenza Vaccine in Solid Organ Transplant (SOT) Recipients	58
12	Lu, Yun Supervisor: Dr. Jeremy Beach	GS	Impact of Introduction of Safety-Engineered Devices on the Incidence of Sharp Object Injury among Health Care Workers in the Capital Region of Alberta	59
13	Deng, Xiaodan Supervisor: Dr. Fabrizio Giuliani	GS	The Role of Rab32 in the Impairment of Mitochondrial Mobility within Neurons: a Possible Cause for Neurodegenerative Processes of Multiple Sclerosis	60
15	Wong, Jonathan Supervisor: Dr. Karen Kroeker	GS	Adherence To Monthly Thiopurine Blood Work Monitoring Declines Over Time In Adults With Inflammatory Bowel Disease	61
16	Nakhaei-Nejad, Maryam Supervisor: Dr. Nadia Jahroudi	PDF	Targeting gene expression to brain and lung vasculature using VWF regulatory sequences	63
17	Shrestha Palikhe, Nami Supervisor: Dr. Lisa Cameron	PDF	The Single Nucleotide Polymorphism, CRTh2-6373G>A, is Associated with Allergic Asthma and Increased Expression of CRTh2	64
18	Yao, Qingxia Supervisor: Dr. Klaus Gutfreund	PDF	Identification Of Programmed Death 1 (Pd-1) And Its Cognate Ligands (Pd-L1, Pd-L2) In The Pekin Duck And Expression Analysis In The Duck Hepatitis B Model	65

Poster Presentations

**Core Internal Medicine & Subspecialty Residents,
Graduate Students & Post Doctoral Fellows**

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22	Paulin, Roxane Supervisor: Dr. Evangelos Michelakis	PDF	Suppressed angiogenesis drives right ventricular failure	69
23	Aldawish, Mohammed Supervisor: Dr. Donald Morrish	PDF	Do low stimulated thyroglobulin levels (<2 ug/L) in differentiated thyroid cancer patients after surgery and radioiodine predict recurrent/persistent disease?	70
24	Chui, Betty Supervisor: Dr. Scott Klarenbach	PDF	Association of pre-dialysis care on mortality and renal outcomes in subjects with chronic kidney disease: a propensity score matched case-control population in Alberta	72
25	Asaduzzaman, Muhammad Supervisor: Dr. Harrisios Vliagoftis	PDF	Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation	73
26	Edgell, Heather Supervisor: Dr. Michael Stickland	PDF	Relationships between cardiovagal baroreceptor sensitivity, peak oxygen consumption, and hypoxia in chronic obstructive pulmonary disease (COPD)	74
27	Haile, Yohannes Supervisor: Dr. Fabrizio Giuliani	PDF	Characterization of NT2 neuronal cell line for the study of inflammation-mediated neurodegenerative processes of multiple sclerosis	75

Poster Presentations

Core Internal Medicine & Subspecialty Residents, Graduate Students & Post Doctoral Fellows

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30	Patel, Aarti Supervisor: Dr. Jack Jhamandas	PDF	“Histamine : a new therapeutic target for Alzheimer’s disease”	78
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33	Meng, Bo Supervisor: Dr. Andrew Mason	PDF	Defining abnormal metabolism in Primary Biliary Cirrhosis	81
34	Patel, Viabhav Supervisor: Dr. Gavin Oudit	PDF	Loss of ACE2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the Ang II/AT1 receptor axis	82
35	Lu, Cathy Supervisor: Dr. Richard Fedorak	CR	Crohn’s Disease Genotype Is Similar Between Patients Who After Discontinuing Infliximab Sustain A Long-Term Remission Versus Those Who Rapidly Relapse	83
36	Weatherald, Jason Supervisor: Dr. Mohit Bhutani	CR	The Impact of Chronic Obstructive Pulmonary Disease on the clinical features of patients with co-existing Heart Failure	84
37	Peerani, Farhad Supervisor: Dr. Richard Fedorak	CR	Crohn's Disease Patients Treated With Infliximab Have Normal Pulmonary Function With Complete Remission But Diminished Pulmonary Function With Partial Response	85

Poster Presentations

**Core Internal Medicine & Subspecialty Residents,
Graduate Students & Post Doctoral Fellows**

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40	Carpenter, Thirza Supervisor: Dr. Steven Katz	CR	Development of a Factor VIII Inhibitor in Association with Giant Cell Arteritis: A Case Report	90
41	Toor, Erinjit Supervisor: Dr. Karen Mulder & Dr. Jennifer Spratlin	CR	Retrospective Analysis Of Outcomes Of Young Patients (Age<40 Years) With Colorectal Cancer (CRC) In Northern Alberta	92
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43	Dhesi, Sumandeep Supervisor: Dr. Daniel Kim	CR	Mechanical Circulatory Support for Cyclophosphamide Induced Cardiomyopathy: A case report and review	94
44	Fan, Xiangning Supervisor: Dr. Jonathan Choy & Dr. Harald Becher	CR	Pixel Intensity: A Novel Method of Quantifying Ejection Fraction in 3D Contrast-Enhanced Echocardiography	95
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Poster Presentations

**Core Internal Medicine & Subspecialty Residents,
Graduate Students & Post Doctoral Fellows**

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50	Chopra, Angeli Supervisor: Dr. Mang Ma	SR	The Utility of Transient Elastography In Monitoring Patients With Wilson's Disease	101
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Scoring Criteria

Oral & Poster Presentations

(1=Poor, 5=Excellent)

Clarity and justification of the research question/hypothesis	1	2	3	4	5
Appropriateness of the methods used to answer the hypothesis	1	2	3	4	5
Validity and relevance of the results to the hypothesis	1	2	3	4	5
Quality of the discussion and conclusion	1	2	3	4	5
Innovation impact	1	2	3	4	5
Visual layout and visual impact	1	2	3	4	5
Oral response to adjudicator's question	1	2	3	4	5

TOTAL SCORE

35

Prioritization and Willingness-to-Pay for Bariatric Surgery: the Patient Perspective

Richdeep S Gill, Sumit R Majumdar, Xiaoming Wang, Rebecca Tuepah, Scott W Klarenbach, Daniel W Birch, Shahzeer Karmali, Arya M Sharma, Raj S. Padwal

INTRODUCTION

Access to publicly funded bariatric surgery is limited, potential candidates face lengthy waits, and no universally accepted prioritization criteria exist. We examined patients' perspectives regarding prioritization for surgery.

METHODS

Consecutively recruited subjects approved for and awaiting bariatric surgery completed a self-administered survey. After reviewing nine scenarios describing hypothetical cases of patients wait-listed for surgery, respondents were asked to rank these hypothetical patients relative to themselves in the surgery queue. Scenarios examined variations in age, clinical severity, functional impairment, social dependence and socioeconomic prominence. Willingness-to-pay for faster access was assessed along a 5-point ordinal scale and analyzed using multivariable logistic regression.

RESULTS

The 99 respondents had mean age 44.7 y (SD 9.9), most (76%) were female, and mean BMI was 47.3 kg/m² (SD 7.6). Mean duration in queue was 34.4 (SD 9.4) months). Respondents assigned similar mean scores (relative to themselves) to hypothetical patients with characteristics identical to themselves ($p=0.22$) and higher mean scores (indicating greater urgency) to those exhibiting greater clinical severity ($p<0.001$) and functional impairment ($p=0.003$). Lower mean scores were assigned to patients at the extremes of age ($p\leq 0.006$), on social assistance ($p<0.001$) and of high socioeconomic prominence ($p<0.001$). 85% of respondents disagreed that payment to expedite access should be available and 67% disagreed with paying for faster access for themselves. Compared to those making $< \$50,000/\text{year}$, respondents making $\$50,000-79,999/\text{year}$ and (adjusted OR 0.11; 95% CI 0.03 to 0.46) and $\geq 80,000/\text{year}$ (adjusted OR 0.16; 95% CI 0.04 to 0.65) were less likely to disagree with paying for faster access for themselves.

CONCLUSIONS

Most wait-listed patients consider greater clinical severity and functional impairments related to obesity to be important prioritization indicators for bariatric surgery and disagreed with others paying for faster access. These findings may help inform future efforts to develop acceptable prioritization strategies for publicly funded bariatric surgery.

Supervisor: Dr. Raj S. Padwal

Mitochondrial Activation Suppresses Hypoxia-Inducible Factor 1 α Signaling in Cancer

Alois Haromy, Peter Dromparis, Trevor H. Stenson, Gopinath Sutendra and Evangelos D. Michelakis

INTRODUCTION

HIF1 α (hypoxia inducible factor-1 α) is activated by hypoxia and increases the expression of pro-angiogenic genes. HIF1 α is signaled for proteosomal degradation by hydroxylation through prolyl-hydroxylases (HPHs), a process requiring both oxygen and the mitochondrial Krebs' cycle intermediate α -ketoglutarate (α KG). Intriguingly, even in normoxia, cancer cells have activated HIF1 α , suggesting that primary inhibition of HPHs in cancer may be due to decreased α KG production. Cancer mitochondria are suppressed and activating mitochondria with the pyruvate dehydrogenase (PDH) kinase (PDK) inhibitor dichloroacetate (DCA) decreases cancer growth in vitro and in vivo. In a small clinical trial with DCA in glioblastoma there was evidence of DCA inhibiting HIF1 α and angiogenesis in vivo. We hypothesized that promoting mitochondrial activity in cancer will increase α KG and inhibit HIF1 α .

METHODS

Non-small cell lung cancer (nSCLC) cells were exposed to 0.5mM DCA for 48 hours. Isocitrate dehydrogenase activity (IDH; the enzyme that produces α KG in the Krebs' cycle) and α KG levels were measured by ELISAs. HPH activity was assessed by HIF hydroxylation using immunoblots. HIF1 α activity was assessed by a dual luciferase reporter assay. PDK and PDH were silenced with siRNA.

RESULTS

By increasing delivery of acetyl-CoA into the Krebs' cycle, DCA increased mitochondrial respiration, IDH activity, α KG levels, HPH activity and decreased HIF1 α activity; its effects were negated by direct HPH inhibitors (CoCl₂ and DMOG). PDK-siRNA and an exogenous, cell-permeable α KG analogue (octyl- α KG) mimicked DCA. PDH silencing did not alter HIF1 α activity in nSCLC, suggesting that it is maximally inhibited in cancer, but silencing PDH activated HIF1 α in normal lung epithelial cells, in which PDH is active.

CONCLUSIONS

We provide a novel mechanism by which a primary mitochondrial dysfunction can inhibit HIF1 α even in the absence of hypoxia and that this is reversed by DCA, potentially explaining clinical evidence that DCA inhibits cancer angiogenesis.

Supervisor: Dr.Evangelos Michelakis

Influence of IL-25 on Th2 Lymphocytes and Allergic Inflammation

Graeme Bredo, Alexis Adams, Jessica Storie and Lisa Cameron

INTRODUCTION

Allergic responses are mediated by inflammatory cells such as Th2 (T helper 2) lymphocytes. CRTh2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) is a marker for Th2 cells and activation through CRTh2 stimulates expression of cytokines important for allergic responses such as IL-4, IL-5 and IL-13. Th2 cell differentiation and expression of CRTh2 is mediated by the transcription factor GATA3. Our lab observed that Th2 cells express the receptor for IL-25, a cytokine shown in animal models to mediate allergic responses. We hypothesize IL-25 plays a role in allergic inflammation by mediating human Th2 cell differentiation, expression of CRTh2 and Th2 cell chemotaxis.

METHODS

IL-25R expression was identified using microarray, qRT-PCR and flow cytometry. The effect of IL-25 on Th2 differentiation was investigated with human CD4 T cells isolated and cultured with anti-CD3/anti-CD28 and IL-2 in Th2 conditions (IL-4, anti-IL-12 and anti-IFN γ) in the presence or absence of IL-25. For chemotaxis assays, CRTh2⁺ cells were isolated and propagated on cycles of activation (anti-CD3/anti-CD28 and IL-2, 3 days) and rest (IL-2, 4 days) and movement determined using modified boyden chamber assay.

RESULTS

A 300-fold higher expression of IL-25R mRNA was observed in CRTh2⁺ Th2 cells, compared to freshly isolated CD4 T cells ($p < 0.05$). Flow cytometry showed highest expression of IL-25R was after activation. CD4 T cell differentiation in the presence of IL-25 resulted in higher Th2 cytokine mRNA and protein as well as GATA3 mRNA expression. IL-25 showed a moderate chemotactic profile.

CONCLUSIONS

These findings show abundant IL-25R expression on CRTh2⁺ Th2 cells and indicate IL-25 mediates Th2 cell differentiation and chemotaxis. IL-25 may play an important role in the allergic inflammatory response in vivo. Our future work will involve studying receptor signaling and other mediators induced by IL-25 from Th2 cells.

Supervisor: Dr. Lisa Cameron

Influence of Cholesterol Accumulation in a Mouse Model of Alzheimer's Diseases

Mahua Maulik 1, 2, 3, Bibaswan Ghoshal 4, John Kim 2, Yanlin Wang 2, 4, Jing Yang², David Westaway 1, 2, 3 and Satyabrata Kar 1, 2, 3, 4

INTRODUCTION

Assimilated evidence suggests that altered levels and/or distribution of cholesterol by regulating β -amyloid ($A\beta$) production from amyloid precursor protein (APP) can influence Alzheimer's disease (AD) pathogenesis. However, very little is known how cholesterol can influence features other than $A\beta$ peptides in AD pathology. We have addressed this issue in a new line of transgenic mice generated by crossing cholesterol accumulating Niemann-Pick type C1 (Npc1)-deficient mice with mutant APP transgenic (APP-Tg) mice. Thus, the objectives of this study were to determine the influence of intracellular cholesterol accretion on survival, behavioral and neuropathological abnormalities and whether these changes can be reversed by treatment with β -cyclodextrin, a compound known to mediate redistribution of the sequestered cholesterol.

METHODS

Transgenic and littermate control mice at 4-, 7- and 10-weeks were used to evaluate their object recognition memory and motor functions. Subsequently, mice were decapitated and their brains were processed using immunohistochemistry and western blotting to determine the neuropathological changes. A subset of mice was treated either with β -cyclodextrin (4gm/kg body weight) or saline at 7 days of age and their behavioral and neuropathological changes were assessed.

RESULTS

1) Intracellular cholesterol accretion in mutant APP-Tg mice can significantly increase mortality rate, trigger early object recognition memory and motor impairments, exacerbate glial pathology, accelerate degeneration of neurons and influence phosphorylation and cleavage of tau protein. 2) Additionally, enhanced levels/activity of cytosolic cathepsin D together with cytochrome c and Bax2 suggest a role for the lysosomal enzyme in the degeneration of neurons. 3) Reversal of cholesterol sequestration by β -cyclodextrin treatment not only increased the longevity but also ameliorated associated behavioral and neuropathological abnormalities in mutant APP-Tg mice.

CONCLUSIONS

Our results suggest that intracellular cholesterol accumulation can influence longevity and a spectrum of behavioral and neuropathological abnormalities associated with AD, which can be significantly reversed by β -cyclodextrin treatment.

Supervisor: Dr. Satyabrata Kar

Hypoxia exposure results in upregulation of VWF gene and its de novo activation in lung microvascular endothelial cells

Anahita Mojiri, Wei-Lee Phan, Steve Kulak, Evangelos Michelakis, Nadia Jahroudi

INTRODUCTION

VWF is expressed exclusively in endothelial cells (EC) and megakaryocyte and its expression is regulated by specific activators and repressors including YY1 and NF1. VWF expression in lung is predominantly detected in larger vessels with low or non-detectable levels in the capillaries. Diseases that target vasculature of distinct organs, such as lung vasculature in pulmonary hypertension, underscore EC heterogeneity. Changes in VWF expression are associated with such diseases.

METHODS

We have previously generated transgenic mice expressing LacZ gene under the regulation of VWF gene sequences that target expression to brain and lung vasculature exclusively. To induce pulmonary hypertension, these transgenic mice were placed in hypoxia chamber. Expression and localization of VWF, LacZ, NFIB and YY1 were evaluated by qRT-PCR and immunofluorescence. Lung microvascular endothelial cells (LMEC) in culture were also exposed to hypoxia and subjected to similar analyses as well as chromatin immunoprecipitation (CHIP) to determine the interaction of NFIB and YY1 to VWF promoter.

RESULTS

In hypoxic transgenic mice, we demonstrated: (i) upregulation of the exogenous and endogenous VWF promoter activity in brain and lung, (ii) redistribution of endogenous VWF and exogenous LacZ transgene expression from primarily larger vessels in the lung to microvessels, and (iii) de novo activation of the exogenous VWF promoter in the heart EC.

Hypoxia-exposed LMEC showed increased VWF expression accompanied by a reduction in association of NF-IB repressor with the VWF promoter, while activator YY1 association was increased. Additionally, increased translocation of YY1 to the nucleus of EC both in vitro and in vivo was associated with hypoxia.

CONCLUSIONS

Hypoxia results in upregulation of VWF promoter activity as well as its de novo activation in microvasculature of lung, suggesting a phenotypic shift from anticoagulant to procoagulant activity in microvascular EC of the lung. This process is associated with modulation of NFIB and YY1 activities.

Supervisor: Dr. Nadia Jahroudi

1H-NMR analyses of brain tissue and cerebrospinal fluid reveal distinct metabolomic profiles: a new strategy to diagnosing and understanding multiple sclerosis.

S.N. Reinke, D.I. Broadhurst, B.D Sykes, W. Branton, G.B. Baker, I. Catz, K.G. Warren, C. Power

INTRODUCTION

Multiple sclerosis (MS) is a debilitating disease of the central nervous system (CNS) affecting as many as one in five hundred Canadians. Metabolomics is the quantitative study of the complement of low molecular weight chemicals (metabolites) in a given biological system. Metabolites as the final downstream products of gene expression enable the provision of highly sensitive phenotypic signatures of disease etiology, clinical presentation, and pathophysiology. In this study we investigate the metabolomic profiles of cerebrospinal fluid (CSF) and brain tissue.

HYPOTHESIS: There are complementary metabolomic signatures of disease in CSF and brain tissue that can facilitate the diagnosis of MS and understanding of MS pathogenesis.

METHODS

1H-NMR spectroscopy was applied to CSF and brain specimens from MS (n=40) and non MS (n=20) patients. Multivariate statistical analysis was performed to discriminate between the two groups, and define a metabolic signature of MS. Changes in metabolite abundance were compared to a deep sequencing database of MS and non-MS brain transcriptomes.

RESULTS

45 metabolites were quantified in CSF, and 30 metabolites in brain tissue. For CSF, a distinct metabolomic signature was uncovered which discriminated MS from non-MS patients (Figure 1) with an area under ROC curve of 0.97 (p<0.001). Profiles of patients with different phenotypes of MS (relapsing-remitting, progressive, and clinically isolated syndrome) were also distinct. Brain data revealed significantly altered concentrations of alanine, lactate, succinate, fumarate, isoleucine, leucine, and valine in MS patients. Interrogation of brain transcriptomes showed decreased expression of succinate dehydrogenase and branched chain α -ketoacid dehydrogenase, and increased expression of alanine transaminase in MS patient's brains.

CONCLUSIONS

These data reveal that patients with MS can be distinguished from non-MS based on their CSF metabolomic profile. MS patients can also be stratified based on their clinical phenotype. In addition, our data suggest that oxidative metabolism is a critical component of MS pathogenesis that may be amenable to future therapeutic interventions.

Supervisor: Dr. Christopher Power

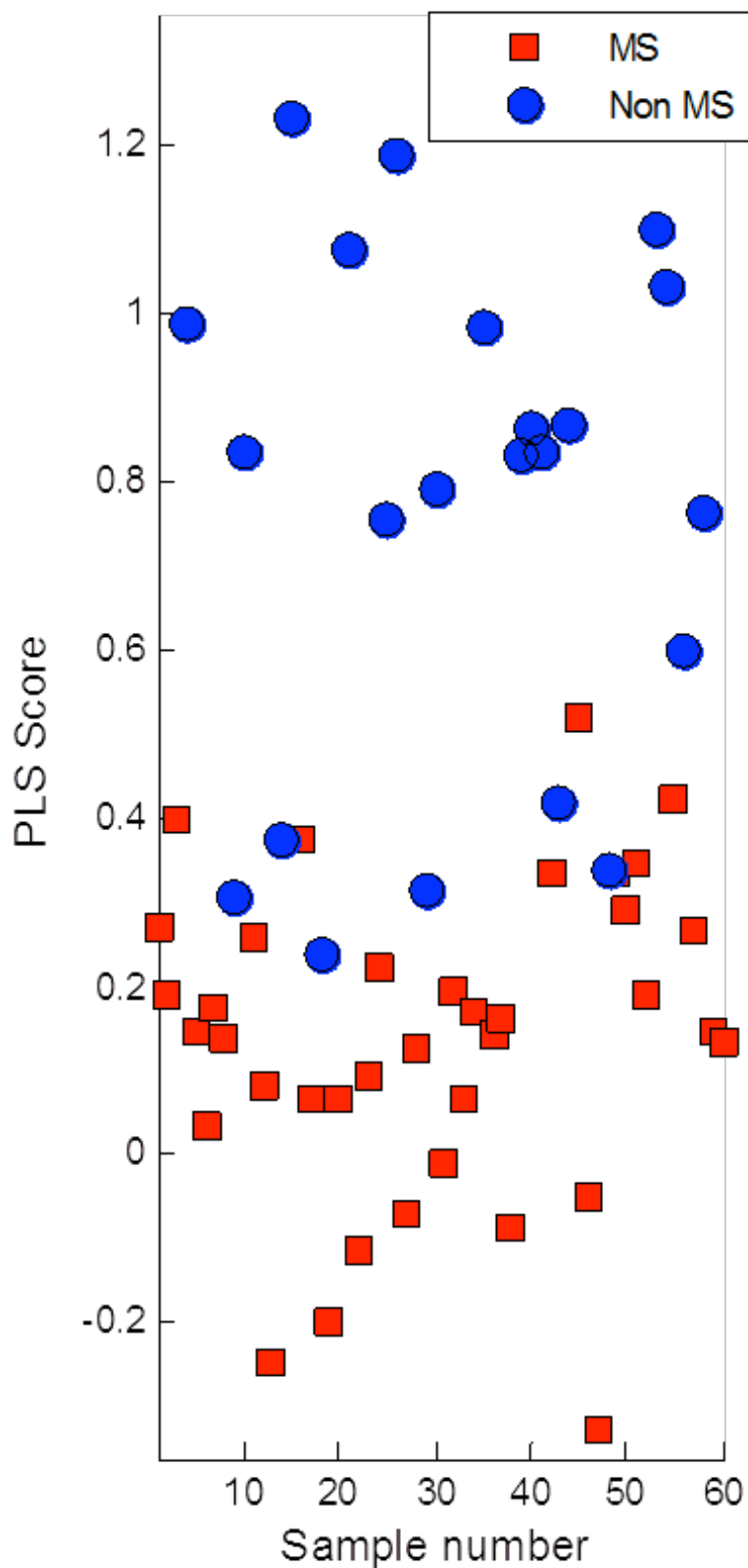


Figure 1. Score plot for a PLS-DA model of CSF metabolomes derived from MS and non-MS patients. Red, MS; blue, non-MS.

Enteropathogenic Escherichia coli (EPEC)-induced epithelial hyposecretion is associated with decreased membrane localization of CFTR

Ohland CL, DeVinney R, MacNaughton WK

INTRODUCTION

EPEC infection causes severe diarrhea in humans, leading to substantial infant mortality in developing countries. The mechanisms of infection are well characterized, including the formation of a type III secretion system (TTSS) to inject bacterial effector proteins into the host cell. However, little is known about how EPEC affects intestinal epithelial ion and water secretion. We hypothesized that EPEC decreases epithelial ion secretion via alteration of ion channel function.

METHODS

The human intestinal cell line T84 was exposed to log phase EPEC at a multiplicity of infection of 100 for 4 hr. Ion transport was measured in Ussing chambers as change in short circuit current. Protein levels of the cystic fibrosis transmembrane conductance regulator (CFTR) were measured by Western blot and densitometry. Membrane localization was determined by cell surface protein biotinylation followed by Western blot.

RESULTS

EPEC significantly decreased ($p < 0.05$) stimulated cAMP-dependent ion secretion (control, 124.7 ± 7.2 ; EPEC, 81.1 ± 14.1 $\mu\text{Amp}/\text{cm}^2$), EPEC mutants lacking the TTSS ATPase or bacterial pore proteins ($\Delta\Delta\text{escN}$, ΔescC , ΔescV) caused similar hyposecretion, indicating that translocation of effector proteins was not required. EPEC mutants lacking the TTSS filament proteins (ΔespA , ΔespB , ΔespD) partially reversed hyposecretion, while a mutant lacking all three (ΔespABD) completely reversed it. Furthermore, a double mutant lacking both TTSS ATPase and flagellin ($\Delta\text{escN}\Delta\text{fliC}$) did not cause significant hyposecretion. This suggests that, in the absence of functional TTSS, filament proteins cooperatively interact with epithelial cells via the flagellar assembly system to cause decreased ion secretion. EPEC exposure also significantly decreased apical localization of CFTR (64.1% of uninfected control). As CFTR was not detectable on the basolateral membrane, we conclude that this channel is being mislocalized to the cytosol, resulting in hyposecretion.

CONCLUSIONS

In conclusion, EPEC decreases cAMP-dependent ion secretion of T84 cells by reducing levels of apical membrane CFTR, which is associated with TTSS filament and flagellum expression.

Supervisor: Dr. Karen Madsen

Inflammasome Expression within the Brain

John G. Walsh, Christopher Power

INTRODUCTION

The chronic inflammation observed in neurodegenerative diseases such as multiple sclerosis (MS) and HIV-associated neuro-cognitive disorders (HAND) has long been associated with the expression of the pro-inflammatory molecules Interleukin-1 β and Interleukin-18. The activation and release of these molecules depends on the formation of the inflammasome complex. However, the presence of the inflammasome in the brains of persons with neuro-inflammatory diseases remains to be fully explored. Herein, we investigated the expression of the inflammasome components within the frontal cerebral white matter from HAND, MS and Other Disease Control (ODC) patients, as well as within primary human neural cell cultures representing the principle cells of the brain.

METHODS

Samples of cerebral white matter from, MS, HAND or ODC patients were taken from autopsied tissues held in the Neurological Infections and Immunity Brain Bank at the University of Alberta. cDNA taken from these samples or from cultured cells was analyzed by semi-quantitative RT-PCR. Primary microglia, astrocytes and neurons obtained from human brain tissue were stimulated in order to activate the inflammasome. IL-1 β production and release was measured by ELISA and western blot.

RESULTS

mRNA expression of inflammasome components (ie. Caspase-1, NLRP3) as well as targets (IL-1 β) were significantly increased in the brains of MS and HAND patients. Analysis of expression within primary neural cells disclosed that microglia but not astrocytes or neurons expressed many components of the inflammasome. Microglia were also able to respond to activators of the inflammasome such as LPS and ATP. With particular relevance to HIV infection of the brain, viral protein gp120 was also found to activate IL-1 β release and maturation in primary human microglia.

CONCLUSIONS

The present observations emphasize the expression of the inflammasome in the human brain and highlight the role of microglia as the chief cellular effector of the brain's inflammasome.

Supervisor: Dr. Christopher Power

ISOLATION OF THE HUMAN BETARETROVIRUS AND DEMONSTRATION OF INTEGRATION SITES IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

W. Wang¹, S. Wasilenko¹, S. Indik², G. Wong¹, A. Mason¹

INTRODUCTION

A human betaretrovirus resembling the mouse mammary tumor virus has been cloned from biliary epithelium and lymph nodes of patients with primary biliary cirrhosis (PBC). We have now isolated the virus from PBC patients' lymph nodes based on co-culture with the Hs578t cell line. To address the hypothesis that a human betaretrovirus (HBRV) is central to the etiology of PBC, we investigated whether viral infection could be detected at the site of disease by identifying proviral integration sites.

METHODS

Biliary epithelium cells (BECs) were extracted from liver sample of patients undergoing transplantation. The presence of HBRV was identified using QuantiGene or by in situ hybridization (ViewRNA). HBRV was isolated in vitro by co-culture of lymph node homogenates from PBC patients with Hs578T cells. DNA was extracted from biliary epithelium, positive Hs578t cell lines and perihepatic lymph nodes. Integration sites were detected using linker mediated-PCR (LM-PCR). LM-PCR products were assessed using paired-end deep sequencing (Illumina Hi-Seq).

RESULTS

HBRV integration sites were detected in 7/9 PBC BEC vs. 1/10 BEC from patients with other liver diseases using LM-PCR ($p < 0.01$) and a similar proportion of PBC patients had evidence of integration sites in their peri-hepatic lymph nodes with 13/15 in PBC vs. 2/14 in other liver diseases. To date, we identified 2698 unique integration sites from all positive lymph node samples, about 684 from the 8 positive cell line libraries. Using in situ hybridization, we observed HBRV in a similar proportion of BEC that had integrations sites.

CONCLUSIONS

The viral isolation studies and detection of viral integration sites in the human genome provide proof that patients with PBC have infection with a transmissible betaretrovirus. The majority of PBC patients tested by linker mediated-PCR to date have evidence of viral infection.

Supervisor: Dr. Andrew Mason

The role of serpina3n as a potentially novel inhibitor molecule in granzyme B-mediated neuronal injury

Haile Y.*, Carmine-Simmen K.***, Kerr B.***, Bleackley C**. and Giuliani F*.

INTRODUCTION

In inflammatory-mediated neurodegenerative diseases such as multiple sclerosis (MS), activated T cells release a serine proteinase-Granzyme B (GrB) and induce neurodegeneration. Serpina3n is a serine proteinase inhibitor isolated in the mouse Sertoli cells. It has been reported that serpina3n prevents GrB-mediated killing of target cells. Therefore, this study aims to investigate the potential role of serpina3n in preventing neuronal injury and in inhibiting the induction/reducing the severity of the disease in an animal model of MS, EAE.

METHODS

Serpina3n was expressed in Jurkat cell lines. Supernatant contained serpina3n was collected and applied on human fetal neurons (HFNs). In addition HFNs were cultured alone, co-cultured with unactivated or anti CD3-activated T cells. The neuronal viability was assessed by immunocytochemistry for MAP-2. Proteins were isolated and the expression and cleavage of alpha-tubulin was evaluated by Western Blotting. EAE was induced and mice were treated with 50 ug serpina3n before and/or at the onset of the disease.

RESULTS

The viability of neurons was significantly low ($28 \pm 12\%$ and $30 \pm 13\%$ respectively) when HFNs were co-cultured with activated T cells, indicating an effective T cell-mediated neurotoxicity. Interestingly, neuronal killing was significantly reduced when activated T cells were pretreated with serpina3n. Indeed, neuronal viability raised to $60 \pm 15\%$. Western Blotting showed the cleavage of the cytoskeletal protein alpha-tubulin when neurons were co-cultured with activated T cells. Nevertheless, alpha-tubulin cleavage was absent when the activated T cells were pre-treated with serpina3n. In vivo, serpina3n delayed the onset and reduced the severity of the disease in EAE mice.

CONCLUSIONS

Activated T cells induce neuronal injury by destabilizing the cytoskeletal microtubules. This effect was reversed when T cells were pretreated with the GrB-inhibitor serpina3n. Moreover, serpina3n delayed and reduced the progression of EAE. These data suggest serpina3n as a potentially novel therapeutic strategy to treat inflammatory-mediated neurodegenerative diseases such as MS.

Supervisor: Dr. Fabrizio Giuliani

Percutaneous Coronary Intervention (PCI) Outcomes of South Asians with Acute Coronary Syndromes

Kendeep S. Kaila MD, Colleen M. Norris PhD, Michelle M. Graham MD FRCPC, Kevin R. Bainey MD FRCPC

INTRODUCTION

People of South Asian (SA) descent are particularly susceptible to coronary artery disease (CAD) with altered outcomes in Acute coronary syndromes (ACS). Percutaneous coronary intervention (PCI) has become a common revascularization strategy in ACS yet the implications on this high-risk population is unknown. Accordingly, we compared long-term clinical outcomes of SA and European Canadians with ACS undergoing PCI.

METHODS

Using the Alberta Provincial Project for Outcomes Assessment in Coronary Heart Disease (APPROACH) we selected patients from January 1995 – December 2009 with ACS undergoing PCI. Information on demographics, comorbid illness, clinical presentation, treatment (including type of stent) and follow up were collected. We evaluated long-term survival and clinical outcomes in patients of SA and European ethnicity using a validated surname selection software with a propensity-score matching technique.

RESULTS

7047 subjects, 1422 SA and 5625 propensity matched European controls, were identified. One-year mortality was similar in both groups (3.6% SA versus 3.9% European; $p=0.59$ respectively). Long-term survival showed a strong trend toward reduced mortality in SA (OR 0.75 $p=0.059$). Differences in one-year mortality were seen in SA with bare metal stents (BMS) and drug eluting stents (DES) (3.8% versus 1.8%), as well as in Europeans with BMS and DES (4.0% versus 1.8%) $p=0.01$). Similar trends were seen with repeat revascularization in SA with BMS and DES (13.1% versus 7.9%) as well as Europeans with BMS and DES (11.2% versus 9.0%) ($p=0.01$).

CONCLUSIONS

Despite prior work demonstrating lower quality of life in SA patients with coronary disease, a trend toward improved survival amongst SA after ACS with PCI is seen when compared to Europeans using propensity-matched controls. Additionally, DES seems to offer benefit in both ethnic groups. Further research is required to understand the apparent disconnect between survival and quality of life in SA patients.

Supervisor: Dr. Kevin R. Bainey

EARLY EXPERIENCE USING A NEW CORE EUS BIOPSY NEEDLE FOR THE DIAGNOSIS OF SOLID MASS LESIONS

Pernilla D'Souza, Gurpal Sandha, Clarence Wong, Safwat Girgis, Christopher Teshima

INTRODUCTION

Endoscopic ultrasound (EUS) is the preferred method to biopsy mass lesions within, and in proximity to, the gastrointestinal tract. EUS-guided biopsies are performed using fine needle aspiration (FNA), which obtains tissue samples for cytology. Recently, a new technique of fine needle biopsy (FNB) was designed to obtain core samples for histology, in the hopes of improving the diagnostic yield and accuracy of EUS-guided biopsy, and an initial case series with this new needle has been promising. We sought to perform a randomized, comparative trial to compare the new ProCore™ (FNB) needle to the standard FNA needle.

METHODS

A prospective study involving 3 endosonographers at the University of Alberta and the Royal Alexandra Hospital was initiated in which all intra-abdominal mass lesions were biopsied using both FNB and FNA needles, with randomization to the first needle used. The primary outcome is the “sample adequacy” for diagnostic interpretation as determined by a single, blinded pathologist. The secondary outcome is the diagnostic yield for positive and/or suspicious biopsies as read by a general pathologist as part of clinical care.

RESULTS

35 solid mass lesions were biopsied during the study period; average age 64 years, 65% men. The overall diagnostic yield for both positive and suspicious diagnoses from FNA versus FNB needles was 71% and 46% respectively ($p= 0.15$). The combination of both needles yielded a diagnosis in 83% of cases. FNB produced inadequate samples in at least 16 cases (6 blood clots and 10 “low cellularity”). There were no complications.

CONCLUSIONS

Our early experience using the FNB needle has been disappointing; with diagnostic rates considerably lower than the case series data. Ongoing patient recruitment continues and further analyses are pending, including determination of primary outcome via blinded pathologist. These results have prompted a quality assessment of our EUS-biopsy procedures.

Supervisor: Dr. Christopher Teshima

Macrolide Use in the Treatment of Critically Ill Patients with Pneumonia: Incidence, Correlates, Timing and Outcomes

Hoang, HL¹, Sligl WI¹, Eurich DT¹, Malhotra A², Marrie TJ³, Majumdar SR¹

INTRODUCTION

Macrolide antibiotics are commonly used to treat pneumonia despite increasing antimicrobial resistance. Macrolides might also decrease mortality in severe sepsis via non-microbial immunomodulatory properties. We studied the incidence, correlates, timing, and mortality associated with macrolide-based treatment of critically ill patients with pneumonia.

METHODS

We prospectively enrolled a population-based cohort of critically ill adults with pneumonia over two years from 5 Canadian intensive care units (ICU). Data collected included disease severity (APACHE II), pneumonia severity (PSI), and type and timing of antibiotic treatments at presentation. Post hoc, “effective” treatment included receipt of antibiotic(s) to which identified pathogen(s) were susceptible or guideline-concordant therapy if no pathogen(s) were identified. The independent association between macrolide-based treatment and 30-day all-cause mortality was examined using multivariable Cox regression.

RESULTS

The cohort included 328 patients; mean PSI 116, mean APACHE II score 17, and 84% required invasive ventilation. Microbiologic diagnoses were made in 156 (48%) patients; *Streptococcus pneumoniae* (59 [18%]) and *Staphylococcus aureus* (46 [14%]) were most common. One-third (91 [28%]) received macrolide-based treatments, with no significant correlates of treatment except nursing home residence (15% vs. 30% for non-residents [$p=0.02$]). 170/328 [52%] of patients received effective antibiotic therapy within 4 hours of presentation. Overall mortality was 54/328 (16%) at 30-days; 14/91 (15%) in those treated with macrolides vs. 40/237 (17%) for non-macrolides [adjusted hazard ratio (aHR) 0.89, 95%CI 0.48-1.65, $p=0.71$]. Patients who received effective antibiotics within 4 hours of presentation were less likely to die than those whose treatment was delayed (14% vs. 17%, aHR 0.50, 95%CI 0.27-0.94, $p=0.03$).

CONCLUSIONS

Macrolide-based treatment was not associated with lower 30-day mortality among critically ill patients with pneumonia, although receipt of any effective antibiotic within 4 hours was. Based on these results, timely treatment is likely more important than choice of antibiotics.

Supervisor: Dr. Wendy Sligl

Retrospective chart review of patients receiving Azacitidine (Vidaza®) in the Edmonton region of Alberta Health Services

Lauren Bolster, Jeff Patterson, Nancy Zhu

INTRODUCTION

Major landmark trials have been published in the last eight years demonstrating a median survival benefit of nine months for higher-risk myelodysplastic syndrome (MDS) patients treated with azacitidine. The purpose of this review is to objectively measure the outcomes achieved with azacitidine in Edmonton for treatment of both MDS and AML (acute myeloid leukemia), and compare it to published randomized controlled trials.

METHODS

Chart review of all patients administered azacitidine in the Edmonton region from time of drug availability, June 2009 to October 2011. Eligible patients were identified by their attending hematologists. Data collected included age, sex, diagnosis, bone marrow biopsy results, blood counts, transfusions requirements, drug doses, hospitalizations, and death. The data was compared to published data regarding the use of azacitidine in patients with MDS.

RESULTS

Fifty-nine patients were identified for review; two patients were excluded due to insufficient data. Diagnoses for azacitidine administration were: 30 (52%) higher-risk MDS and 27 (47%) AML. Major hematologic improvement was seen in the erythroid cell line in 42%, platelets 42%, and neutrophils in 23% of cases. The overall median survival was 10.5 months; MDS patients was 12.5 months, and AML patients was 5.25 months.

CONCLUSIONS

As expected, AML patients had worse outcomes compared to the MDS cohort, but randomized clinical trial data for comparison is unavailable. Major hematologic improvement including transfusion independence was comparable or better than what is reported in the literature for MDS (erythroid 40%, platelet 33%, neutrophil 19%). A striking difference in median overall survival was noted, with overall survival being nearly half of what is published in the literature for MDS patients (12.5 vs. 24 months). Additional research is needed to understand how to best utilize azacitidine for elderly patients with MDS and AML to maximize its real world efficacy.

Supervisor: Dr. Nancy Zhu

A Population-based study on care and clinical outcomes in remote-dwellers with heavy proteinuria

Aminu Bello, Natasha Wiebe, Brenda Hemmelgarn, Braden Manns, Scott Klarenbach, Stephanie Thompsom, Rick Pelletier, Marcello Tonelli

INTRODUCTION

Patients with proteinuria are at high risk of cardiovascular and renal complications. Since this risk can be reduced by appropriate interventions, we hypothesized that remote-dwellers, who are known to have lower access to health care, might have a higher risk of complications.

METHODS

Using a database of all adults with at least one measure of urine protein between May 2002 and March 2009, we examined the frequency of heavy proteinuria, quality of care delivery and rates of adverse clinical outcomes across travel distance categories to the nearest nephrologist. Heavy proteinuria was defined by albumin:creatinine ratio (ACR) ≥ 60 mg/mmol, protein:creatinine ratio (PCR) ≥ 100 mg/mmol or protein $\geq 2+$ on dipstick urinalysis.

RESULTS

Of 1,359,330 subjects in the study, 262,209 were remote-dwellers. The overall prevalence of proteinuria was 2.3%, 2.9% and 2.5% in those who live >200 km, 100.1-200 km and 50.1-100 km respectively as compared to 1.5% in those who live within 50 km of the nearest nephrologist ($p < 0.001$). Similarly, the prevalence of heavy proteinuria was increased among remote-dwellers compared to urban dwellers ($p = 0.001$ for trend). There were no differences in markers of good quality care or the rate of adverse outcomes (all-cause mortality, heart failure and renal outcomes) across distance categories. However, the rate of hospitalizations and stroke were significantly higher with increased distance from the nearest nephrologist ($p < 0.001$ and 0.02 respectively).

CONCLUSIONS

Heavy proteinuria was common in Alberta residents, especially in remote-dwellers. Care seemed similar across distance categories of travel, but with higher risk of hospitalizations and stroke among remote-dwellers. Further work is needed to understand the basis for the increased risk of hospitalizations and stroke.

Supervisor: Dr. Marcello Tonelli

How common are false positive and false negative findings in cardiovascular meta-analyses?

¹Zaina AlBalawi, ¹Michelle Wong, ²Kristian Thorlund, ¹Anita Chan, ²Jorn Wetterslev, ¹Finlay McAlister

INTRODUCTION

The potential impact of systematic error on meta-analyses is well recognized. Although random error can also lead to false positive or false negative results, its impact is less well appreciated. Trial sequential analysis (TSA) is analogous to the use of interim monitoring boundaries in randomized trials and can be used to account for the risk of random error in meta-analyses.

METHODS

We selected all completed reviews approved by the Cochrane Heart Group and published in the 2011 Cochrane Library that contained at least one meta-analysis of at least 5 randomized trials with binary patient important outcomes. We calculated the optimal information size after adjusting for heterogeneity in each meta-analysis and applied TSA. We classified statistically significant meta-analyses as true positives if their pooled sample size exceeded the heterogeneity-adjusted optimal information size and/or their cumulative Z-curve crossed the O'Brien-Fleming monitoring boundaries for detecting a RRR of at least 25%. We classified meta-analyses which did not achieve statistical significance as true negatives if their pooled sample size was sufficient to reject a RRR of 25%.

RESULTS

Of the 56 eligible meta-analyses published in the 2011 Cochrane Heart Group, 23 (41%) reported statistically significant results and 31 (55%) had sufficient data to be classified definitively positive (19 of the 23 [83%] statistically significant meta-analyses) or definitively negative (12 of the 33 [36%] non-statistically significant meta-analyses). The authors of 10 of the statistically significant reviews concluded that their results demonstrated superiority of the experimental intervention; however, 4 of these meta-analyses were classified as potential false positives on TSA. Thirteen of the non-significant review authors concluded that their results proved no difference between the experimental and control interventions: we found that 8 of these analyses were potential false negatives. Thus, TSA confirmed that 11 (48%) of the 23 meta-analyses which authors asserted were conclusive were truly conclusive.

CONCLUSIONS

Half of the Cochrane meta-analyses we studied had different implications if the risk of random error was taken into account.

Supervisor: Dr. Finlay McAlister

USE OF HYPNOTICS IN HOSPITALIZED CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

Sohaib J, Yehya J, Man GCW, Mayers I

INTRODUCTION

Patients with chronic obstructive pulmonary disease (COPD) frequently complain of sleep disturbances for which hypnotics may be prescribed. In hospitalized medical patients, increases in the length of stay, risk of falls and other adverse effects of hypnotics have previously been reported.

Because of the potential side effects of respiratory depression, American Thoracic Society practice guidelines recommend that “hypnotics should be avoided, if possible, in patients with severe COPD”. However, the pattern of hypnotics use in hospitalized COPD patients and its impact on clinical outcomes are not known.

METHODS

A retrospective hospital chart review was performed for all adult patients with a diagnosis of Acute Exacerbation of COPD (AECOPD). The review was limited to patients admitted to the medical services at the University of Alberta Hospital in the year 2010. Patients with primary diagnosis of other pulmonary diseases, major psychiatric illness and prolonged hospital stay (>30 days) were excluded.

RESULTS

Data from 178 patients were included for analysis (88 males and 90 females). Age was 69.43 ± 12.49 years (mean \pm SD). 102 patients received hypnotics (group H) and 76 did not (group NH). Hypnotic medications prescribed included benzodiazepines (n=60) and non-benzodiazepines (n=67). There was no difference between H and NH in age and BMI. The length of stay (LOS) was 8.94 ± 6.54 days for the entire group. LOS in H was significantly longer than in NH (10.26 ± 6.88 days vs. 7.21 ± 5.54 days; $p=0.001$). Subgroup analysis showed that the difference in LOS was evident irrespective of age group, gender or severity of COPD.

CONCLUSIONS

- 1- A majority of AECOPD patients (57.3%) received hypnotics in the hospital, despite the recommendation against hypnotics use.
- 2- There was a significantly longer LOS (by 3 days) in patients who received hypnotics compared with patients who did not receive hypnotics.

Supervisor: Dr. Godfrey Man

Initial Brain Natriuretic Peptide levels, but not hemodynamics, predict 5-year survival in patients with Pulmonary Arterial Hypertension

Vikram Gurtu, Laura Morrison, Linda Webster, Dale Lien, Evangelos D. Michelakis

INTRODUCTION

Although brain natriuretic peptide (BNP) correlates with prognosis in patients with left ventricular dysfunction, its role in Pulmonary Arterial Hypertension (PAH) is unknown outside of short-term data. The strongest predictor of morbidity and mortality in PAH by far is right ventricular (RV) function. Current PAH therapies do not appear to improve survival. We hypothesized that BNP at the time of referral would predict long-term survival better than hemodynamic parameters available by echocardiography or catheterization and independent of subsequent PAH therapies.

METHODS

PAH patients followed at our PAH program between 2002 and 2011 were included in a registry. A cohort of 72 consecutive patients had BNP on referral (n=72), and survival was followed up to five years while in a subset, BNP, MRI-measured RV mass and catheterization were performed within 24 hours (n=27). Pearson correlations assessed relationships between hemodynamic parameters, BNP, and survival. Kaplan Meier Curves compared survival depending on BNP range.

RESULTS

Among patients with BNP<400pg/mL vs BNP>400pg/mL, cumulative survival at 60 months was 93% vs 23% respectively (Figure). Both BNP and six-minute walk (6MW) test correlated with survival ($R^2=0.45$, $p<0.01$; $R^2=0.1258$, $p<0.02$). However, RV systolic pressure (RVSP) by echocardiography, mean pulmonary arterial pressure (PAPm) and pulmonary vascular resistance (PVR) by catheterization failed to predict survival. BNP correlated with RV mass index, right atrial pressure and 6MW ($R^2=0.588$, $p<0.001$; $R^2=0.477$, $p<0.001$; $R^2=0.403$, $p<0.001$ respectively), but not with PAPm or PVR.

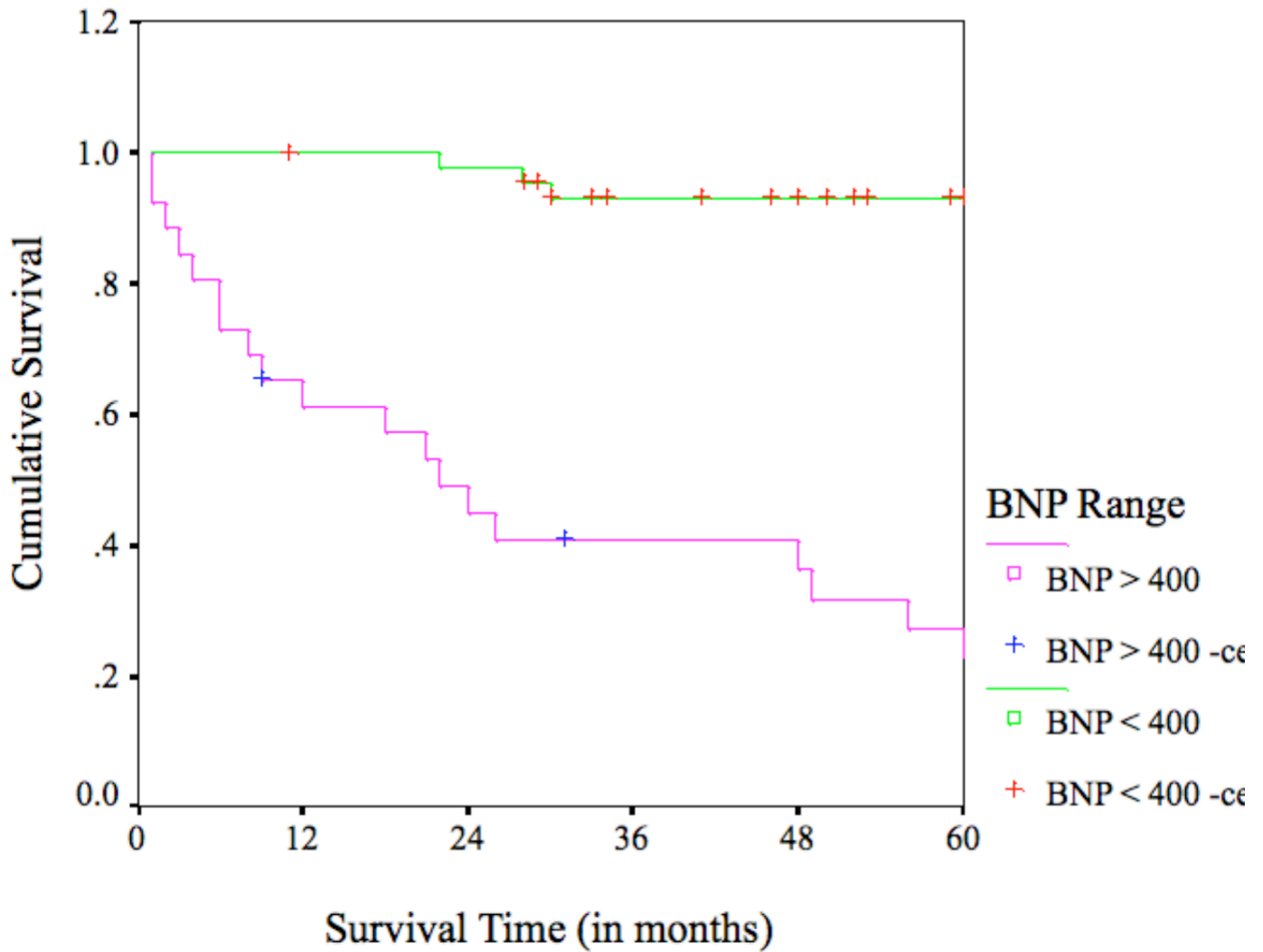
CONCLUSIONS

The predictive power of initial BNP levels on long-term survival in PAH patients and its correlation with RV remodeling suggests that RV function upon referral may determine prognosis regardless of subsequent interventions (all patients were exposed to the same evidence-based therapy protocols). Our data suggest that BNP could potentially be used to alter clinical care, since it could identify patients that need earlier referral for transplantation and intensified/accelerated treatment protocols.

Supervisor: Dr. Evangelos D. Michelakis

Figure 1: Kaplan Meier Survival Statistics

Survival in PAH Patients Based on BNP Range on Referral



α -synuclein levels in the blood as a potential biomarker for presymptomatic PD

Gorostidi A, Bergareche A, Ruiz-Martínez J, Martí-Massó JF, Rodríguez-Oroz M, López de Munain A, El-Agnaf O, Al-Zahmi F.

INTRODUCTION

Diagnosis Parkinson's disease (PD) remains clinical, based on phenotypic patterns. Identifying laboratory biomarkers that are specific for PD will allow preclinical detection, which could play a role in developing treatments designed to slow or prevent progression of the disease. Alpha-synuclein is a key protein in the pathogenesis of PD. Aggregated forms of α -synuclein accumulate in affected brain regions leading to progressive loss of dopaminergic neurons.

METHODS

With the objective of defining the validity of α -synuclein levels as a preclinical or progressive biomarker in an accessible non-invasive human samples, we measured α -synuclein levels in the plasma of idiopathic PD and LRRK2 mutations carriers' patients as well as in asymptomatic mutation carriers compared to healthy control subjects using an enzyme-linked immunosorbent assay (ELISA).

RESULTS

We found a significantly lower level of α -synuclein in plasma of both PD patients (n=166, p=0,006) and in asymptomatic mutation carriers (n=16, p=0,007) compared to aged matched healthy controls. In PD subgroups, idiopathic PD patients had a significant difference (n=134, p=0,009)) however, in LRRK2 mutation carrier's patients, this difference did not reach statistical significance (n=32, p = 0.07). Despite the significant results, no predictive value of total α -synuclein in the diagnosis of PD was observed with the ROC analysis

CONCLUSIONS

PD is associated with lower levels of α -synuclein in the plasma. The decrease in α -synuclein plasma levels may precede the clinical manifestations of the disease, and hence, this may play a role as an early biomarker for PD. This is the first study of α -synuclein analysis to include presymptomatic subjects and therefore, although a preliminary study, we emphasize the importance of plasma α -synuclein measurement in PD risk groups.

Supervisor: Dr. Omar El-Agnaf

Obesity and Outcomes in Patients Hospitalized with Pneumonia

Kahlon SK, Eurich DT, Padwal RS, Malhotra AM, Minhas-Sandhu JK, Marrie TJ and Majumdar SR

INTRODUCTION

Obesity is a risk factor for acquiring pneumonia, but studies also paradoxically suggest it is associated with better pneumonia-related outcomes. We examined the impact of obesity on short-term mortality in patients hospitalized with pneumonia.

METHODS

Clinical data, including Pneumonia Severity Index (PSI), were prospectively collected for 2 years on all consecutive adults admitted with pneumonia to six hospitals in Edmonton, Alberta, Canada. Of these, we identified 907 patients who also had body mass index (BMI, kg/m²) collected. Patients were categorized as underweight (BMI < 18.5 kg/m²), normal (18.5 to < 25 kg/m²), overweight (25 to < 30 kg/m²), and obese (> 30 kg/m²). Multivariable logistic regression analyses, using normal (BMI 18-25 kg/m²) as reference, were undertaken to evaluate the independent association between obesity and in-hospital mortality.

RESULTS

Overall, 65% of the cohort were >65 years, 48% were female, and 15% reported recent weight loss. In terms of BMI, 84 (9%) were underweight, 358 (39%) normal, 228 (25%) overweight, and 237 (26%) obese. Two-thirds had severe pneumonia (63% PSI Class IV/V) and 79 (9%) patients died. In-hospital mortality was greatest among the underweight (12 [14%]) vs normal (36 [10%]) vs overweight (21 [9%]) vs obese (10 [4%], p=0.01). Compared with normal weight, obese patients had lower rates of in-hospital mortality (4% vs 10%, unadjusted odds ratio (OR) 0.39, 95%CI 0.19-0.81) that remained significant in multivariable analyses adjusted for age, sex, comorbidities, and clinical-radiographic severity of pneumonia (adjusted OR 0.44, 95%CI 0.21-0.94, p=0.035). Conversely, compared with normal weight, neither underweight (adjusted p=0.47) nor overweight (adjusted p=0.64) were associated with mortality.

CONCLUSIONS

In patients hospitalized with pneumonia, obesity was independently associated with lower short-term mortality, while neither underweight nor overweight were. This finding suggests a paradoxical relationship between BMI > 30 kg/m² and mortality that requires better mechanistic understanding.

Supervisor: Dr. Sumit Majumdar

Chemical chaperone mediated inhibition of activating transcription factor 6 (ATF6) is a Novel Therapy in Pulmonary Arterial Hypertension

Peter Dromparis, Roxanne Paulin, Adam Kinnaird, Trevor H. Stenson, Alois Haromy Gopinath Sutendra and Evangelos D. Michelakis

INTRODUCTION

The high mortality associated with pulmonary arterial hypertension (PAH) is in part, due to the complex and poorly understood pathogenesis. ER-stress is a potential common denominator among many of the seemingly unrelated molecular triggers of PAH, including mutations and the unfolded protein response that follows viruses, inflammation and hypoxia. The ER forms a functional unit with the mitochondria (the ER-mitochondria unit), allowing for exchange of Ca^{2+} , lipids and ATP.

Recently, we showed that ER-mitochondria unit disruption was critical in PAH pathogenesis. In pulmonary artery smooth muscle cells (PASMCs), ER-stress activated the transcription factor ATF6, which increased levels of the ER-resident protein Nogo, caused a structural/functional disruption of the ER-mitochondria unit, and resulted in the apoptosis-resistance that is central to pulmonary vascular remodeling in PAH. Chemical chaperones including the FDA-approved 4-phenylbutyrate (PBA) attenuate ER-stress and have been studied in metabolic diseases and cancer in animals and patients. We hypothesized that attenuation of ER stress-induced ATF6 activation with PBA will prevent the disruption of the ER-mitochondria unit and prevent/reverse PAH.

METHODS

We use two PAH models associated with ER-stress: hypoxia and monocrotaline. Animals were treated with PBA in their drinking water (~500mg/kg/day) in both prevention (days 0-28) and reversal (days 14-28) protocols. Mechanistic studies were performed in lungs of these animals and murine PASMCs exposed to 48hr hypoxia.

RESULTS

We show PBA decreases ATF6 activation (nuclear colocalization, luciferase, target gene (Nogo/GRP78) expression) and normalizes mitochondrial function (mitochondrial- Ca^{2+} , membrane potential, reactive oxygen species). Both in vivo and in PASMCs in vitro, PBA suppresses proliferation (Ki67) and induces apoptosis (TUNEL). Treated animals had less distal pulmonary artery remodeling, improved hemodynamics, decreased right ventricular hypertrophy and better functional capacity compared to vehicle-treated controls (see table; data presented as mean±SEM).

CONCLUSIONS

Chemical chaperones reduce pulmonary vascular remodeling by inhibiting ATF6 activation, a pathway compatible with several PAH-etologies.

Supervisor: Dr. Evangelos Michelakis

Parameter	Normoxia + Vehicle (n=12)	Normoxia + PBA (Reversal) (n=9)	Chronic Hypoxia + vehicle (n=11)	Chronic Hypoxia + PBA (Reversal) (n=15)	Chronic Hypoxia + PBA (Prevention) (n=12)
mPAP (mmHg)	15.39±0.57	15.62±0.93	35.84±1.32	21.83±0.89	18.99±0.72
TPR (mmHg ml ⁻¹ min)	0.70±0.04	0.73±0.07	2.56±0.16	1.13±0.05	0.92±0.04
PAAT (msec)	26.74±0.99	26.69±1.33	14.70±0.67	19.51±0.99	21.11±0.74
%RV/LV+septum	20.23±0.28	22.11±1.01	34.79±1.43	27.28±1.00	27.34±0.94
Treadmill (m)	503±42	482±57	211±33	362±39	382±50
%MWT	20.86±2.29	21.31±1.96	36.00±2.13	27.14±1.82	26.22±1.41

Patho-physiology of Iron-Overload Cardiomyopathy: Linking Oxidative Stress to Diastolic Dysfunction- improvement with Resveratrol

Subhash K. Das^{1,2} M.Sc, Vaibhav B. Patel^{1,2}, Seyyed M.R. Kazemi-Bajestani^{1,2}, Jason Dyck, PhD and Gavin Y. Oudit^{1,2}, MD, PhD, FRCP(C)

INTRODUCTION

Iron-overloaded cardiomyopathy is a newly recognized cause of heart failure with high morbidity and mortality. Iron-overload in the heart results in iron-induced oxidative stress and organ dysfunction and is associated with genetic as well as acquired iron-overload.

METHODS

We developed a murine model of acquired iron-overload cardiomyopathy to better understand the basic mechanism of diastolic dysfunction in iron-overload conditions and to develop novel therapeutic strategies. We treated 10 week old male C57BL6 mice sub-acutely/chronically with iron-dextran at 5 mg/25g body weight i.p, 5 days/week for either 4 /12weeks. We also examined the effects of the antioxidant and metabolic modulator, resveratrol (190 mg/kg/day), on the iron-overload phenotype. Mice were subjected to echocardiography, hemodynamic studies, molecular and histopathological studies.

RESULTS

Hemodynamic and echocardiographic analysis showed diastolic dysfunction with preserved systolic function in the iron-overload group. Resveratrol treatment prevented the development of diastolic dysfunction. Histopathological staining such as Prussian blue staining conformed iron deposition in cardiac and liver tissue while PSR and Trichome staining showed increased fibrosis in the chronically iron-overload hearts. Inductively coupled plasma mass spectrometry confirmed quantitative iron deposition in the heart and liver. Resveratrol completely prevented the development of myocardial fibrosis. Gene expression analysis of different genes showed a significant up regulation in iron-overload groups and was normalized in the Resveratrol treated groups.

CONCLUSIONS

Iron-overload resulted in oxidative stress mediated selective diastolic dysfunction with preserved systolic function. Treatment with resveratrol can prevent oxidative stress and myocardial injury and protect the heart from the iron-overload induced diastolic dysfunction in patients with iron-overload.

Supervisor: Dr. Gavin Y.Oudit

Oncostatin M Receptor deficiency is associated with less mortality and attenuated multi-organ dysfunction in sepsis

Barbara Pedrycz, Don Grynoch, Catharine Compston, Lin-Fu Zhu, Nick Nation, Rachel Khadaroo, Valerie A. Luyckx, Thomas F. Mueller

INTRODUCTION

Patients with acute kidney injury, especially when associated with multi-organ dysfunction (MOD), have a high mortality rate, basically unchanged over the last 50 years. Inflammation is a key process in kidney injury and MOD. We could demonstrate the crucial role of the Il6-family cytokine Oncostatin M (Osm) and its receptor (Osmr) in the renal inflammatory response. Furthermore, our findings indicate that Osmr-deficient (-/-) mice are protected against distant organ injury.

METHODS

To further understand the role of Osmr in mediating inflammation and injury we induced sepsis in wild type (WT) and Osmr^{-/-} mice by cecal ligation and puncture (CLP) as the prototypic model of systemic inflammation and MOD. Kidneys, livers, hearts, lungs and blood were harvested 24 hours after CLP. Organ injury and function was assessed by real-time RT-PCR, histopathology and immunoassays. Altogether 34 mice were studied with at least 5 mice per condition.

RESULTS

The intensity of sepsis, as induced by increasing size of cecal puncture holes, was reflected by increased transcript levels of organ injury markers and Osmr in WT mice. CLP performed with 18 G needles resulted in a 40% mortality rate in the WT vs. 0% in Osmr^{-/-} mice. WT compared to Osmr^{-/-} mice had a worse kidney function (BUN of 150 vs. 41 mg/dl in WT vs Osmr^{-/-}, $p < 0.0001$), exhibited higher systemic Il6 concentrations (101,700 vs. 22,000 pg/ml in WT vs. Osmr^{-/-}), and showed higher tissue transcript levels of inflammation and injury as measured by Il6 and Ngal (Il6 of 28.3 vs. 5.5 and Ngal of 3,115 vs. 1,416 %Hprt resp. in WT vs. Osmr^{-/-}).

CONCLUSIONS

Overall, our results indicate that Osmr is not only a novel biomarker of organ injury but also a potential therapeutic target to protect against MOD and improve outcomes in syndromes with systemic inflammation.

Supervisor: Dr. Thomas Mueller

Preventing HCV re-infection Post-transplant: In Vitro Evaluation of Novel agents providing Cross Genotype Protection against HCV

Daire O'Shea, MD, John Law, PhD, Adrian Egli, MD PhD, Norman Kneteman, MD, Michael Houghton, PhD, Lorne Tyrrell, MD, Deepali Kumar, MD and Atul Humar, MD

INTRODUCTION

Strategies to prevent HCV re-infection are key to improving outcomes after liver transplantation. Cross neutralising monoclonal antibodies (mAb) targeting HCV E1-E2 have been developed and may inhibit HCV infection. Also, Silibinin, a milk thistle extract, exhibits anti-HCV activity. Silibinin has been used in case reports for prevention of HCV infection in patients undergoing liver transplantation. Silibinin displays antifibrotic, anti-inflammatory and anti-proliferative properties, all potentially advantageous in transplantation. The purpose of this study was to evaluate these novel agents in an in vitro HCV cell culture (HCVcc) model

METHODS

We analysed the activity of antigenic region 3 specific mAbs (AR3A) and silibinin using the JFH-1 HCVcc model. The chimeric viruses display E1-E2 from different genotypes, and encode luciferase reporter genes. Human hepatoma (Huh) 7.5 cells were treated with serial dilutions of silibinin pre-infection. mAbs were pre-incubated with virus construct for 1 hour pre-infection. Luciferase activity expressed as relative fluorescent units (RFU) was measured at 48h postinfection

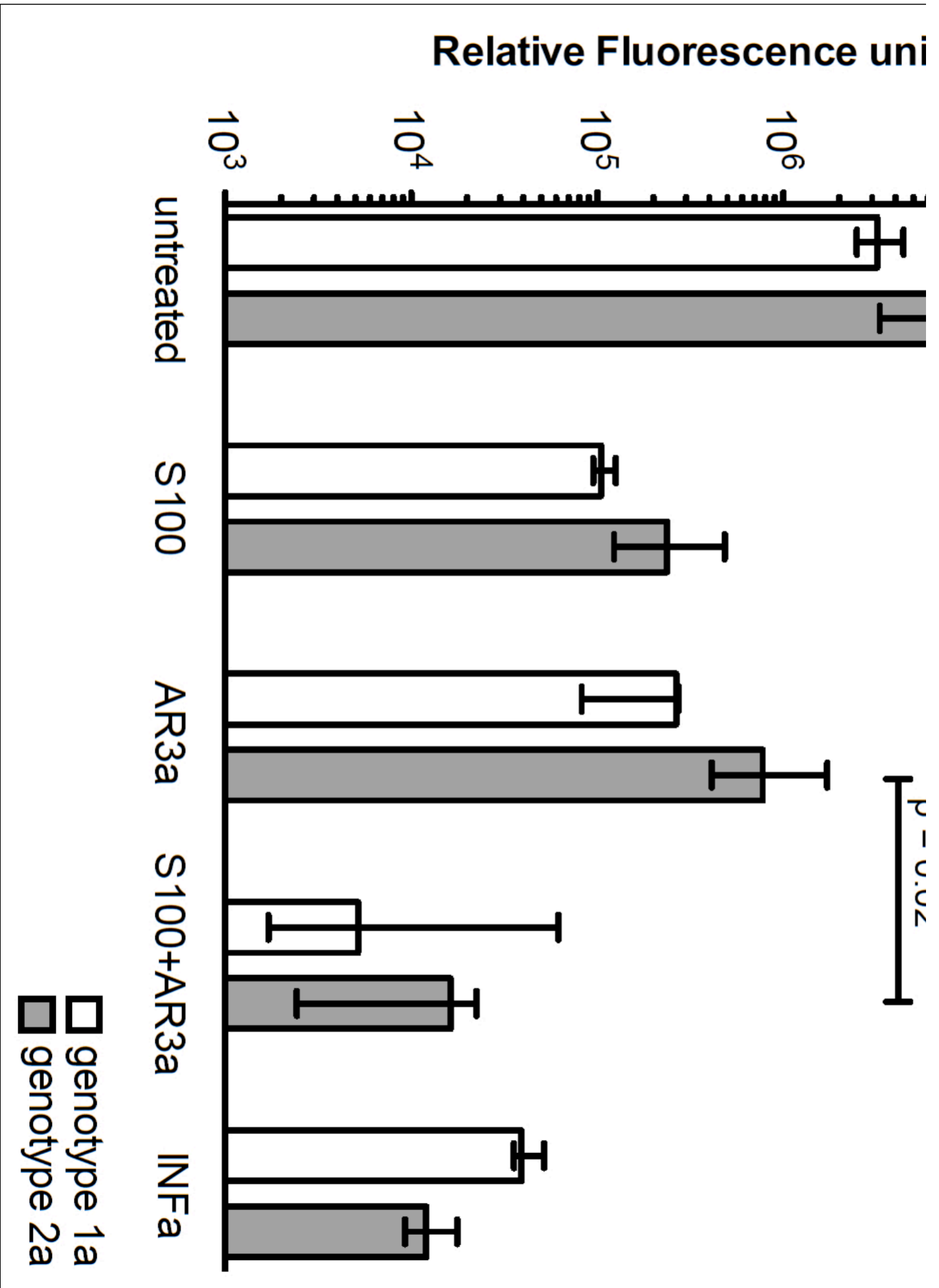
RESULTS

Silibinin and AR3A inhibited HCV(2a) infection of Huh 7.5 cells in a dose-dependent manner. Silibinin 25, 50, and 100 g/ml caused a 0.5, 0.63, and 1.4 log reduction of RFU respectively, compared with untreated cells ($p=0.001$); AR3A mAb (10 g/ml) caused a 1.3 log reduction of RFU ($p=0.003$). Combining the agents resulted in a significant additional reduction in infection compared to either agent alone ($p=0.039$, $p=0.02$ for Silibinin, AR3A respectively). Similar findings were observed with a genotype 1a construct

CONCLUSIONS

We demonstrate strong anti-HCV activity of both AR3A mAb and silibinin in an in-vitro model. In combination these agents significantly reduced HCV infection further. Activity was maintained against different genotype constructs. These novel agents may be an effective and safe means to prevent infection of naive hepatocytes and have potential to prevent HCV reinfection post liver transplant

Supervisor: Dr. Atul Humar



Airborne particulate matter alters intestinal permeability and immune function through direct effects on innate immune cells

Lisa Kish¹, Saad Y. Salim¹, Naomi Hotte¹, Robert Tso¹, Caroline D. Ethier¹, Renaud Vincent², Gilaad G. Kaplan³, Herman Barkema³, Karen Madsen¹

INTRODUCTION

Exposure to airborne pollutants is a risk factor for the development of respiratory and cardiovascular disease. Although recent epidemiology studies have shown an association between airborne pollutants and increased risk of appendicitis and inflammatory bowel disease, little is known as to how airborne particulate matter (PM) affects the gut. The aim of this study was to examine the effects of orally ingested PM on gut immune function and permeability.

METHODS

Wild type (WT) mice were gavaged for 7 or 14 d with Ottawa urban PM (360ug EHC-93) or vehicle. Mucosal immune function was assessed by cytokine secretion from cultured tissue as measured by MesoScale Discovery platform. Gene expression in small (SI) and large (LI) intestine was measured with Taqman low density arrays. Ingenuity pathway analysis was used to identify specific pathway interactions. Gut permeability was measured by urinary excretion of lactulose and mannitol. In vitro effects of PM were studied in HT-29 and T84 (epithelial) and THP-1 (macrophage) cell lines. Permeability in HT-29 and T84 cells was measured by FITC-dextran translocation. PM effects on cytokine secretion and E. coli uptake and killing by THP-1 cells were measured.

RESULTS

WT mice treated with PM showed significant alterations in gene expression and cytokine secretion. In the SI, genes associated with immune cell development and function were differentially expressed in PM-treated mice, with additional differences in gene expression related to cellular signaling and trafficking appearing by d14. This was associated with enhanced secretion of IL1B, IL12, CXCL1, IL2, IL10, IFN γ , and TNF α and increased gut permeability at d7. However, by d14, cytokine secretion had returned to normal with the exception of IFN γ and IL-10 secretion which were decreased, suggesting PM-induced alterations in T cell function. In the LI, genes associated with immune responses and immune cell trafficking were differentially expressed by d7, with further differences in gene expression related to cellular development, maintenance, and cell mediated immune responses appearing by d14. Increased IFN γ and decreased IL-10, TNF α , and IL-2 secretion were seen by d14. In vitro experiments showed that treatment of HT-29 and T84 cells with PM did not alter permeability. In contrast, PM treatment of THP-1 cells induced IL-1B secretion and decreased THP-1 cell ability to uptake and kill E. coli.

CONCLUSIONS

Orally ingested airborne pollutants elicit an initial increase in gut permeability and acute inflammatory response that is followed by subsequent alterations and depression of intestinal immune function. These effects appear to be due to a

direct effect of PM on gut innate immune cells. These findings provide a mechanism whereby airborne PM may play a role in the triggering or exacerbation of gastrointestinal disease.

Supervisor: Dr. Karen Madsen

CLIC5A Maintains Ezrin-Dependent Podocyte Structure and Function

Abass Al-Momany, Laiji Li and Barbara J. Ballermann

INTRODUCTION

Podocytes extend interlocking actin-based extensions (foot processes, FP) around the exterior of kidney glomerular capillaries, forming a buttress that maintains capillary integrity under the high filtration pressure. Podocyte injury leads to diabetic nephropathy. We identified CLIC5A as a podocyte-predominant protein. Here, we studied the mechanism of CLIC5A function.

METHODS

Confocal immunofluorescence (cIF) and immunogold transmission electron microscopy (IG-TEM) were performed in CLIC5 deficient mice and wild-type littermates. Diabetes was induced with streptozotocin. Injury was assessed by urinary protein excretion and histopathology. CLIC5A, GFP-CLIC5A, or vector control, were transiently overexpressed in Cos-7 cells, normally null for CLIC5A.

RESULTS

In wild-type mice, CLIC5A co-localized with ezrin in podocytes. IG-TEM showed highly polarized CLIC5A localization at the apical plasma membrane of FPs, similar to ezrin. In glomerular lysates from CLIC5 deficient mice, and by cIF of glomeruli, total ezrin was dramatically reduced compared to WT mice.

Phospho-(Thr567)-ezrin (p-ezrin), required for the ezrin-actin interaction, was abundant in podocytes of WT, but absent in CLIC5A deficient mice. In diabetic CLIC5A deficient mice there was significantly greater glomerular injury (proteinuria and glomerulosclerosis) than in controls.

In total cell lysates of Cos-7 cells CLIC5A overexpression significantly increased p-ezrin abundance ($n = 3$), without change in total ezrin. This increase in p-ezrin led to its translocation to the cytoskeletal fraction. Live-cell image analysis with the phosphatidylinositol 4,5-bisphosphate (PIP2) reporter RFP-PH-PLC showed its translocation to discrete patches projecting from the apical cell membrane in cells transfected with CLIC5A. RFP-PH-PLC, the PIP2 kinase HA-PIP51K and actin all co-localized with GFP-CLIC5A in these apical projections.

CONCLUSIONS

Absence of CLIC5A predisposes to podocyte injury and diabetic nephropathy. By translocating the PIP2 kinase, CLIC5A enhances apical PIP2 generation leading to localized ezrin phosphorylation and re-organization of actin-based cellular projections. Hence, we believe we have described a key mechanism in the formation of podocyte foot processes which, if absent, predisposes to podocyte injury.

Supervisor: Dr. Barbara J. Ballermann

Predictors of A1c among women with a history of gestational diabetes in Aboriginal communities of Alberta

Richard T Oster, Ellen L Toth

INTRODUCTION

In the Canadian Aboriginal population (First Nations, Métis, and Inuit), gestational diabetes (GD) contributes to a vicious cycle by increasing the risk of type 2 diabetes in both offspring and mothers. Given the growing evidence of the ability of hemoglobin A1c (A1c) to forecast future diabetes, we sought to examine the predictors of A1c among non-diabetic women (mostly Aboriginal) with a history of GD in the province of Alberta.

METHODS

We accessed the databases of three separate community-based diabetes and risk screening projects. A total of 184 adult (≥ 20 years) women with previous GD were screened with clinical exams and portable lab technology between the years 2003-2011. Of these women, 114 were First Nations, 40 were Métis, and 30 were non-Aboriginal. A1c, body mass index, waist circumference, fasting or random glucose, total cholesterol, high-density lipoprotein cholesterol, blood pressure, as well as the presence of metabolic syndrome and family history of diabetes was assessed. Analysis of variance and chi-square tests were used to identify any between group (ethnicity) differences. Statistical modeling using multiple regression analysis was conducted to quantify the relationships between A1c and measured variables.

RESULTS

In the final adjusted model, significant associations remained only for waist circumference (beta coefficient 0.018; 95% CI 0.007 - 0.031; $p = 0.02$) and age (beta coefficient 0.018; 95% CI 0.010 - 0.026; $p < 0.001$). In other words, for a one unit increase in waist circumference, A1c increases by 0.018 in the model (on average). Likewise, for a one unit increase in age, A1c increases by 0.018 (on average). Ethnicity was not associated with A1c.

CONCLUSIONS

Increasing waist circumference and age are predictive of A1c among women with previous GD.

Supervisor: Dr. Ellen L Toth

Dietary adequacy of vitamin D and calcium among Inuit and Inuvialuit women of child-bearing age in Arctic Canada: A growing concern

Fariba Kolahdooz, Alison Barr, Cindy Roache, Tony Sheehy, Andre Corriveau, Sangita Sharma

INTRODUCTION

Arctic populations are at an increased risk of vitamin D inadequacy due to geographic latitude and a nutrition transition. This study aimed to assess the adequacy of dietary vitamin D and calcium among women of child-bearing age in Arctic Canada.

METHODS

A cross-sectional survey of 203 randomly selected women of child-bearing age (19-44 years) was conducted in six remote communities in Nunavut and the Northwest Territories of Arctic, using a validated quantitative food frequency questionnaire. Data were analyzed to determine dietary adequacy of vitamin D and calcium, to summarize the top foods contributing to vitamin D and calcium intake among traditional food eaters (TFE) and non-traditional food eaters (NTFE).

RESULTS

The response rate was between 69-93% depending on the community sampled. Mean BMIs for both TFE and NTFE were above the normal range. Traditional food eaters had a significantly higher mean vitamin D intake compared with non-traditional eaters (TFE=7.11±7.47µg/day; NTFE=4.88±4.72µg/day, p=0.004). The majority of women (87%) were below the Estimated Average Requirements (EARs) for vitamin D. Despite adequate mean daily calcium intake in both TFE and NTFE (TFE=1428±649mg/day; NTFE=1120±565mg/day, p=0.0005), 27% of the study population fell below the EAR for calcium. Dairy products contributed the most to intake of vitamin D (TFE=30.7%; NTFE=39.1%) and calcium (TFE=25.5%; NTFE=34.5%).

CONCLUSIONS

Inadequate dietary vitamin D intake is evident among Inuit and Inuvialuit women of child-bearing age in Arctic Canada. Promotion of nutrient-rich sources of traditional foods, supplementation protocols and/or expanded food fortification should be considered to address this nutrition concern.

Supervisor: Dr. Sangita Sharma

Potential role of IGF-II receptor in APP processing

Yanlin Wang^{1,2}; Satyabrata Kar^{1, 2, 3}

INTRODUCTION

The insulin-like growth factor-II (IGF-II) receptor involves in the transport of newly synthesized lysosomal enzymes from the trans-Golgi network to endosomes. The endosomal-lysosomal system, the major site of IGF-II receptor expression, plays a critical role in the processing of amyloid precursor protein (APP) leading to the generation of β -amyloid ($A\beta$) peptide - a key player in the development of Alzheimer's disease pathology. However, the role of the receptor in APP processing remains unclear. To address this issue we used IGF-II receptor overexpressing and deficient fibroblast cell lines to study the influence of the receptor on APP processing and $A\beta$ metabolism.

METHODS

IGF-II receptor overexpressing and deficient cells were cultured in DMEM and then processed using PCR-arrays to detect the mRNA levels of markers of APP processing and endosomal-lysosomal system. Subsequently, we used western blotting, fluorometric kits and/or ELISA to measure levels of APP, its processing enzymes and $A\beta$ peptides. Pepstatin A, a specific inhibitor for the lysosomal enzyme cathepsin D, was used to verify its role in APP processing.

RESULTS

PCR-array data revealed higher mRNA levels of APP as well as β - and γ -secretases in IGF-II receptor overexpressing cells. In accordance with PCR-array data, we observed increased levels of APP holoprotein, β -C-terminal APP fragment (the immediate precursor of $A\beta$). Secreted N-terminal APP fragment, $A\beta$ 1-40 and $A\beta$ 1-42 were higher in the conditioned media of IGF-II receptor overexpressing cells. Increased expression and activities of β -secretases and cathepsin D are also found in IGF-II receptor overexpressing cells. Additionally, pepstatin A inhibits the generation of β -C-terminal APP fragment without affecting the levels of APP and related β - and γ -secretases.

CONCLUSIONS

These results suggest that higher levels of IGF-II receptors increase levels of APP and the expression and activity of β -secretases leading to increased generation of $A\beta$ peptides probably through increasing cathepsin D expression/activity.

Supervisor: Dr. Satyabrata Kar

TIMAP Regulates Akt Phosphorylation and Endothelial Cell Proliferation

Marya Obeidat, Laiji Li and Barbara Ballermann

INTRODUCTION

Angiogenesis is the process by which new blood vessels form by sprouting of pre-existing vessels. This process is crucial for normal and pathological vascularization. Proliferation of endothelial cells (EC) is a major step in angiogenesis. VEGF and TGF β 1 are essential regulators of EC angiogenesis. We identified TIMAP (TGF β 1 Inhibited Membrane Associated Protein) in EC. TIMAP is EC-predominant regulatory subunit of protein phosphatase 1 (PP1c). We have shown that TIMAP can inhibit PP1c activity towards certain proteins. Here, we show that TIMAP downregulation inhibits EC proliferation and Akt phosphorylation. Akt phosphorylation is stimulated by VEGF and vital for EC angiogenesis. Hence, Identifying the role of TIMAP in VEGF-mediated angiogenesis will help develop new therapies to target pathological angiogenesis in tumors and retinal angiopathies.

METHODS

EC were transfected with control siRNA or TIMAP specific siRNA. Proliferation of EC was examined using flow cytometry analysis of EdU incorporation into the DNA of proliferating cells. Real-Time records of cell growth were obtained using Electrical Cell Impedance Sensing (ECIS) system. Electrical impedance increases in proportion to the increase in cell number. Protein phosphorylation was detected by western blot. EC were treated with VEGF and the association between TIMAP and PP1c was detected by Co-immunoprecipitation.

RESULTS

Inhibition of TIMAP decreased EdU incorporation by 40% compared to control, $p=0.03$, $n=3$.

The rate of electrical impedance in TIMAP depleted cells was 15x lower compared to control, $p=0.03$, $n=4$.

TIMAP depleted cells exhibited 7 fold reduction in Akt phosphorylation, $p=0.006$, $n=4$.

VEGF enhanced the co-immunoprecipitation between TIMAP and PP1c.

CONCLUSIONS

TIMAP is a positive regulator of EC proliferation and Akt phosphorylation.

VEGF enhances the interaction between TIMAP and PP1c.

Hence, we propose that VEGF-stimulated interaction between TIMAP and PP1c inhibits PP1c-mediated dephosphorylation of Akt, and that in the absence of TIMAP PP1c activity is unleashed resulting in dephosphorylation of Akt, possibly leading to inhibition of EC proliferation.

Supervisor: Dr. Barbara Ballermann

A Randomized Trial of High-dose Intradermal versus Intramuscular Seasonal Influenza Vaccine in Solid Organ Transplant (SOT) Recipients

Aliyah Baluch, MD, Atul Humar, MD, Adrian Egli, MD, PhD, Katja Hoschler, PhD, Patricia Campbell, MD, Hamid Liacini, PhD, Nouredine Berka, PhD, Simon Urschel, MD, Leticia Wilson, MSc and Deepali Kumar, MD

INTRODUCTION

Organ transplant recipients are at high-risk of influenza related complications. However, standard Intramuscular (IM) vaccine has a suboptimal response in this population. Intradermal (ID) influenza vaccine, places the antigen in a site of a higher density of dendritic cells and may increase vaccine immunogenicity. We evaluated high-dose ID vaccine compared to IM in SOT patients.

METHODS

During the 2010-2011 influenza season, SOT patients were randomized to receive standard-dose IM or high-dose ID influenza vaccine. Sera collected pre- and 4 weeks post-immunization were used to perform strain-specific antibody titer using the hemagglutination inhibition assay (HAI). Seroprotection was defined as a post-vaccination titer 1:40 and seroconversion was a 4-fold rise in titer from baseline.

RESULTS

We enrolled 229 transplant patients including kidney (n=94), lung (n=74), liver (n=26), heart (n=18) and combinations (n=17). Demographics including age, transplant type, time from transplant, and immunosuppression were similar in both groups. Overall, 212 patients were evaluable (105 IM, 107 ID). Seroconversion to at least one vaccine strain was 46.7% and 51.4% in the IM and ID groups respectively (p=0.5). Seroprotection rates to A/H1N1, A/H3N2, and B were 70.5%, 63.8%, and 52.4% in the IM group versus 71.0%, 70.1% and 63.6% in the ID group respectively. If lung transplants were excluded, recipients of high-dose ID vaccine had greater seroprotection rates (p=0.032), and seroconversion factors (p=0.013) to influenza B. On multivariate analysis, independent predictors of vaccine response were time post-transplant >6 months and mycophenolate mofetil dose <2g. Both vaccines were safe and there was no clinically significant production of de-novo donor specific alloantibody.

CONCLUSIONS

We showed that both high-dose ID and standard-dose IM influenza vaccines demonstrated similar immunogenicity in SOT patients. However, ID vaccine responses were greater for influenza B in the non-lung subgroup suggesting a preferential role for use of this vaccine in some transplant types.

Supervisor: Dr. Deepali Kumar

Impact of Introduction of Safety-Engineered Devices on the Incidence of Sharp Object Injury among Health Care Workers in the Capital Region of Alberta

Yun Lu, Ambikaipakan Senthilselvan, Mark Joffe, Jeremy Beach

INTRODUCTION

The use of safety engineered devices became widespread in 2007-2008 in many Alberta health care facilities due to changes in health and safety regulations requiring their use. The aim of this study was to investigate the frequency of sharp object injuries and to determine the effectiveness of safety-engineered devices in preventing these injuries among health care workers in Alberta's Capital Region facilities.

METHODS

A retrospective cohort of all sharp object injuries reported to Alberta Health Services (AHS)/Edmonton Zone Workplace Health and Safety offices from staff working in health care facilities between 2003-2010 was undertaken. These data were anonymised and recorded in two separate databases (EPINET and MedGate) over this period. Rates of sharp object injury among health care workers were compared before (2006), during (2007 - 2008), and after (2009 - 2010) the introduction of safety-engineered devices, adjusting for other potential risk factors. Poisson regression/log-linear models were used for statistical analyses.

RESULTS

During 2003 to 2010, a total of 4047 sharp object injuries were reported from 15 health care facilities in the Capital Region. Nurses reported the largest proportion of sharp object injuries (53.7%), followed by physicians (27.7%). The sharp object injury rate per FTEs/year during the introduction period declined to 30.17 compared with 34.47 before the introduction period (rate ratio [RR]: 0.88, 95% CI: 0.78, 0.99). Nurses showed the greatest decrease (RR =0.85, 95% CI: 0.74-0.97). Physician rates showed little change during the period of introduction (odds ratio (OR): 0.99, 95%CI: 0.85-1.14) but decreased significantly after the intervention (OR =0.83, 95%CI: 0.71-0.97) in comparison to the period before the intervention.

CONCLUSIONS

The introduction of safety-engineered devices was associated with a reduction in sharp object injuries among health care workers, however this appeared to be relatively short-lived except among physicians.

Supervisor: Dr. Jeremy Beach

The Role of Rab32 in the Impairment of Mitochondrial Mobility within Neurons: a Possible Cause for Neurodegenerative Processes of Multiple Sclerosis

Xiaodan Deng, Diane Turner, Thomas Simmen, Fabrizio Giuliani

INTRODUCTION

In multiple sclerosis (MS), mitochondrial mobility and redistribution following demyelination is impaired and make cells more susceptible to inflammatory insult, causing neurodegeneration and subsequent progression of the disease. Recent studies indicated a role for Rab32, a member of the Ras protein family, in mitochondria mobility. We investigated whether or not the decreased mitochondrial mobility in neurons is potentially caused by mutation and/or over-expression of Rab32. In addition, we measured the levels of Rab32 in MS brain and non-inflammatory control brain.

METHODS

Following transfection using Amaxa Basic Nucleofector Kit Primary Neurons, SKH cells were grown on coverslips for 24 hours, then incubated with mitotracker red, fixed in 4% paraformaldehyde and washed with IF washing buffer. Samples were mounted in Prolong AntiFade. Images were obtained with an AxioCam on an Axio Observer microscope and enhanced with Adobe Photoshop using the levels functions only.

Frozen brains samples from an MS brain and non-inflammatory control (amyotrophic lateral sclerosis) brain were added to extraction buffer and sonicated by 550 Sonic Dismembrator. Protein concentrations were measured by NanoDrop Spectrophotometer ND1000 at 280nm. Western blotting was performed on both extracts using goat-anti mouse/rabbit secondary antibodies conjugated with Alexafluor 680/750 on an Odyssey infrared imaging system.

RESULTS

Western blotting results indicated that Rab32 is expressed in both MS and non-inflammatory control brains. Rab32 levels were slightly decreased in the inflammatory brain compared to the non-inflammatory control brain.

Transfection of dominant-negative Rab32 mutant was performed on SKH neuronal cell line and data demonstrated that mutation and over-expression of Rab32 caused abnormal mitochondrial clustering within neuronal cells.

CONCLUSIONS

The decreased levels of Rab32 in the MS brain tissue and the disruption of mitochondrial dynamics within neuronal cells following Rab32 mutation or over-expression are suggestive for a potential role of Rab32 in neurodegenerative process of MS. Further experiments are currently ongoing to confirm our findings.

Supervisor: Dr. Fabrizio Giuliani

ADHERENCE TO MONTHLY THIOPURINE BLOOD WORK MONITORING DECLINES OVER TIME IN ADULTS WITH INFLAMMATORY BOWEL DISEASE

Jonathan G Wong, Richard N Fedorak, Karen Wong, Karen I Kroeker

INTRODUCTION

Patient adherence to medical therapy is usually studied in the context of medication use. Thiopurines, such as azathioprine (AZA) and 6-mercaptopurine (6-MP), are frequently used to maintain remission in inflammatory bowel disease (IBD) patients. However, due to the adverse effects of thiopurines, namely leukopenia and elevated liver enzymes, monthly blood work monitoring is recommended to patients on thiopurines. The aim of this study is to determine the adherence of patients on thiopurines to monthly blood test monitoring and to determine if this changes over time on thiopurines.

METHODS

A patient search was conducted in a university gastroenterology patient database to identify IBD patients currently on thiopurines. Charts were reviewed for demographic information, diagnosis and blood test frequency and results, which include white blood cell (WBC) and liver enzymes. Data was recorded on a spreadsheet. For the purpose of this study, only blood tests measured over 20 days apart were counted as monthly blood tests. In order to standardize results, blood work was grouped based upon the time period from initiating thiopurine therapy. Results were subsequently categorized into groups by number of venipunctures per year. Data was analyzed using Microsoft Excel and SPSS.

RESULTS

The initial patient search identified 348 IBD patients on thiopurines. 165 patients were included in this study. Reasons for exclusion include: did not live in the local area and so could not confirm if all blood tests were available (163); patients were in clinical trials (4); and incomplete patient records (16). Mean age was 36.5 ± 1.1 years, 48% were male, and 76% had Crohn's disease. The average blood tests per year (mean \pm SE) for the first year of thiopurine treatment was higher than patients on thiopurines for greater than one year (7.7 ± 0.32 v. 5.3 ± 0.17 , $p = 5.47 \times 10^{-11}$).

CONCLUSIONS

IBD patients on thiopurines had blood tests less frequently than the recommended monthly monitoring. Adherence to blood work monitoring was significantly higher in the first year of thiopurine treatment compared to subsequent years.

Supervisor: Dr. Karen I. Kroeker

Table 1. Frequency of venipuncture per year varies by duration of thiopurine use (Data reported as number and % of patients in that year of thiopurine use; Chi-squared, $p=0.000015$)

Year of Thiopurine	Annual # Venipuncture				
	0	1-3	4-6	7-9	10-12
1 (n=78)	0	4 (5.1%)	23 (39.5%)	28 (35.9%)	23 (29.5%)
2 (n=59)	0	15 (25.4%)	24 (40.7%)	9 (15.3%)	11 (18.6%)
3 (n=55)	0	14 (25.5%)	21 (38.2%)	13 (23.6%)	7 (12.7%)
4 (n=54)	0	17 (31.5%)	21 (38.9%)	10 (18.5%)	6 (11.1%)
5 (n=50)	1 (2%)	14 (28.0%)	23 (46.0%)	8 (16.0%)	4 (8.0%)
6+ (n=65)	2 (3.1%)	20 (23.3%)	31 (27.7%)	11 (16.9%)	1 (1.5%)

Targeting gene expression to brain and lung vasculature using VWF regulatory sequences.

Maryam Nakhaei-Nejad, Anahita Mojiri, Steve Kulak, Allan Murray, Nadia Jahroudi

INTRODUCTION

Von Willebrand factor (VWF) is an endothelial-specific protein that is expressed differentially in various vascular beds. We have identified regions of VWF promoter that target activation to distinct organs' vasculature. Using transgenic mice, we identified distinct regions of the VWF gene that are necessary for promoter activation in vasculature of brain only (sequences -487 to +247), or lung and brain (I51-HSS plus -487 to +247). To determine whether organ-specific characteristics of VWF promoter are maintained when delivery vectors for somatic targeting of endothelial cells are used, we generated adenovirus containing LacZ gene under the regulation of lung-brain specific VWF promoter. Additionally, we are exploring the possibility that endothelial progenitor cells (EPCs) can be modified *ex vivo* to express desired transgene prior to *in vivo* delivery, in a manner that targets transgene expression to specific vascular beds.

METHODS

Mice injected with adenovirus containing the LacZ gene under the lung-brain specific VWF sequences (Adn-I51HSS) were euthanized and analyzed for expression of LacZ gene in various organs. To test the feasibility of transgenically modifying EPC *in vitro* and detecting the transgene expression *in vivo*, EPCs were transduced with adenovirus containing lacZ under CMV ubiquitous promoter. Mice injected with these transduced EPCs were euthanized and analyzed for LacZ expression.

RESULTS

Adn-I51HSS injection resulted in specific expression of LacZ in endothelium of lung and brain, exclusively. CMV-LacZ adenovirus transduced EPCs injected to normal mice could be detected in lung, brain, heart and kidney but not liver vasculature.

CONCLUSIONS

We have demonstrated that organ specific activities of VWF sequences are maintained using adenovirus targeting system, thus allowing delivery of therapeutic targets to specific organs' vasculature. Further, incorporation of EPCs, which were manipulated *in vitro* before administration, into vasculature of various organs could provide a useful mechanism of combining gene and cell therapy approaches for vascular bed-specific manipulation.

Supervisor: Dr. Nadia Jahroudi

The Single Nucleotide Polymorphism, CRTh2-6373G>A, is Associated with Allergic Asthma and Increased Expression of CRTh2

N. Shrestha Palikhe, E. Campos Alberto, E. MacLean, C. Davidson, J. Storie, D. Brenner, A. El-Sohemy, H. Vliagoftis, L. Cameron

INTRODUCTION

CRTh2 (chemoattractant-receptor homologous molecule expressed on Th2 cells) is expressed by Th2 cells and other cells involved in allergic inflammation. The single nucleotide polymorphisms (SNPs) in CRTh2 (rs11571288, 545659, 634681) have been associated with phenotypes of allergic disease in ethnically distinct populations, but no CRTh2 SNPs have been shown to influence endogenous CRTh2 expression.

METHODS

The promoter SNP, CRTh2-6373G>A (rs533116), was genotyped in an ethnically diverse population (n=1282). Flow cytometry was used to assess the proportion of circulating peripheral blood cells expressing CRTh2 in subjects with allergic airways disease and controls as well as in vitro differentiated Th2 cells. Receptor function was assessed by Th2 cytokine expression in response to a CRTh2-specific agonist DK-PGD2 with intracellular staining and Elisa.

RESULTS

CRTh2-6373G>A was associated with allergic asthma in Caucasians (OR 2.67, $p < 0.01$) and expression of CRTh2 was higher in subjects with allergic airways disease compared to controls ($p < 0.05$). Amongst allergic individuals, -6373G>A was associated with significantly more eosinophils and higher expression of CRTh2 by both CD4⁺ T cells and eosinophils ($p < 0.05$). In vitro, the A allele coincided with a higher percentage of CD4⁺ T cells expressing CRTh2 under Th2 differentiating conditions and the expression of IL-4 and IL-13 following DK-PGD2 stimulation ($p < 0.05$).

CONCLUSIONS

These findings suggest CRTh2-6373G>A influences susceptibility of developing allergic asthma, through higher expression and responsiveness of CRTh2. Therapeutics targeting CRTh2 are now in clinical trials and so further study is needed to determine whether genotyping for CRTh2-6373G>A would be useful for developing a personalized approach to treating allergic disease

Supervisor: Dr. Lisa Cameron

IDENTIFICATION OF PROGRAMMED DEATH 1 (PD-1) AND ITS COGNATE LIGANDS (PD-L1, PD-L2) IN THE PEKIN DUCK AND EXPRESSION ANALYSIS IN THE DUCK HEPATITIS B MODEL

Yao Q, Fischer KP, Tyrrell DL and Gutfreund KS

INTRODUCTION

Programmed death 1 (PD-1, CD279) negatively regulates TCR complex-initiated signaling by interacting with its cognate ligands (PD-L1 and/or PD-L2). PD-1 is highly expressed on exhausted T cells and blockade of this pathway has been shown to restore antiviral immunity in persistent viral infections. The aim of this study was to identify and characterize the duck homologues duPD-1, duPD-L1 and duPD-L2 and to explore the role of PD-1 signalling in the immunopathogenesis of duck hepatitis B virus (DHBV) infection.

METHODS

The complete open reading frame of duPD-1 and partial open reading frames (>90%) of duPD-L1 and duPD-L2 were obtained by RT-PCR and RACE on RNA isolated from duck splenocytes. DuPD-1, duPD-L1 and duPD-L2 transcript levels were assessed by real-time PCR in PBMCs and tissue of DHBV naïve animals and liver of ducks infected with DHBV.

RESULTS

The predicted 283 amino acid (aa) duPD-1 protein has an identity of 70%, 32% and 31% with chicken, murine and human PD-1 proteins, respectively. The predicted duPD-L1 and duPD-L2 proteins had an aa identity of approximately 80% and 40% with chicken and human homologues, respectively. DuPD-1 transcripts were predominately expressed in thymus, spleen and bursa, and mirrored expression levels of duPD-L1 and duPD-L2 in these tissues. Higher expression levels of duPD-L1 were observed in lung. Mitogen stimulation of PBMCs increased duPD-1 and duPD-L1 mRNA expression levels, whereas duPD-L1 and duPD-L2 expression also increased during culture in the absence of mitogens. Expression patterns of duPD-1, duPD-L1 and duPD-L2 were similar in liver tissue of DHBV-naïve and DHBV-infected animals (congenital and acute-resolved infection).

CONCLUSIONS

The identification of duPD-1 and cognate ligands will facilitate further studies of PD-1 signaling in the immunopathogenesis of DHBV infection. The development of reagents suitable for expression analysis on isolated intrahepatic and peripheral T cells and other tissue resident cells can now be pursued and these reagents may prove useful for strategies that aim to enhance antiviral immunity through blockade of PD-1 signalling.

Supervisor: Dr. Klaus S. Gutfreund

IDENTIFICATION OF THE PEKIN DUCK INTERLEUKIN-10 RECEPTOR-2

Yao Q, Fischer KP, Tyrrell DL and Gutfreund KS

INTRODUCTION

Interleukin-10 receptor 2 (IL-10R2) is a shared cell surface receptor required to exert the biological activities of several class 2 cytokines that play critical roles in host defence. Several of these cytokines, such as IL-10, IL-22, IL-28 and IL-29 have been implicated in the immunopathogenesis of hepatitis B. We have recently identified duck IL-10 (duIL-10) and its isoform that lacks helix F (duIL-10 Δ 5) and the duck IL-10R1 receptor chain. The aim of this study was to identify and characterize duck IL-10R2 (duIL-10R2) and the duck IL-10 receptor complex with the long-term goal to study the role of immunomodulatory cytokines in the immunopathogenesis of acute and chronic duck hepatitis B virus (DHBV) infection.

METHODS

The open reading frame of duIL-10R2 was obtained by RT-PCR with primers based on chicken sequence and 5' RACE from duck splenocytes. IL-10R2 transcript levels were assessed by real-time PCR and normalized to GAPDH.

RESULTS

Sequence analyses revealed an open reading frame encoding a 343 amino acid protein. The predicted protein showed an identity of 76%, 42% and 43% with chicken, human and murine homologues, respectively. The mature duIL-10R2 protein has similar structure to chicken and mammalian species. DuIL-10R2 transcripts were widely expressed in primary and secondary immune organs, lung, liver and kidney. In freshly isolated peripheral blood mononuclear cells (PBMCs) duIL-10R2 mRNA was readily detected. During prolonged culture IL-10R2 transcripts increased in the absence of mitogens. Mitogen stimulation resulted in a marginal transient increase of duIL-10R2 transcripts followed by a decline to levels below those of control treated cells.

CONCLUSIONS

DuIL-10R2 has significant homology with mammalian and chicken homologues. The identification of the duIL-10 receptor complex will allow the study of the role of the identified duIL-10 isotype proteins in receptor binding and signaling. This may also facilitate the evaluation of strategies that block IL-10 signaling to enhance T cell effector function in proof of concept immunotherapeutic studies and the identification of other class 2 cytokine receptors that share the IL-10R2 common chain in the duck model of chronic hepatitis B infection.

Supervisor: Dr. Klaus S. Gutfreund

Patients with inflammatory bowel disease exhibit dysregulated responses to microbial DNA

Saad Y Salim, PhD1, Naomi Hotte, Robert H Tso, BSc1, Phil Bach, MD, John Walker, PhD, MD, Levinus A Dieleman, MD1, Richard N Fedorak, MD1, Karen L Madsen, PhD1

INTRODUCTION

A critical role for the gut epithelium lies in its ability to discriminate between pathogens and commensals and respond appropriately. Dysfunctional interactions between microbes and epithelia are believed to have a role in inflammatory bowel disease (IBD). In this study, we analyzed microflora and gene expression in IBD patients and examined responses of mucosal biopsies to bacterial DNA.

METHODS

Biopsies were taken from non-inflamed areas of the colon in healthy controls (HC) and Crohn's disease (CD) and ulcerative colitis (UC) patients. Biopsies were snap-frozen or cultured with DNA from *Lactobacillus plantarum* (LP) or *Salmonella dublin* (SD). Gene expression was analyzed under basal conditions and in response to DNA. Gene networks were analyzed using Ingenuity Pathways. Mucosal-associated microflora was analyzed using terminal restriction fragment length polymorphism. Frequency of single nucleotide polymorphisms in NOD2 and TLR9 was assessed.

RESULTS

Patients with IBD had altered microflora, enhanced expression of inflammatory mediators, and increased correlations between specific gene expression and microbes. Principle component analysis showed CD patients to cluster independently from UC and HC based upon their microflora. DNA from LP stimulated anti-inflammatory pathways in controls and UC patients, but induced an upregulation of IL17A in CD patients. There were no differences in SNP frequencies of TLR9 or NOD2 in the groups.

CONCLUSIONS

Crohn's disease patients exhibit altered responses to bacterial DNA. These findings suggest that the gut response to bacterial DNA may depend not only on the specific type of bacterial DNA, but also on the luminal environment of the host.

Supervisor: Dr. Karen L Madsen

Regulation of HIV-1 in the brain: the contribution of microRNAs

Hui E, and Power C

INTRODUCTION

Regulation of gene expression by microRNAs (miRNAs), predicated on complementary base-pairing of targeted messenger RNAs, has made miRNAs appealing candidates for monitoring disease progression. We hypothesized that select cellular miRNAs influenced human immunodeficiency virus 1 (HIV-1) gene expression by targeting HIV-1 genes.

METHODS

Analyses of microRNA arrays comparing RNA derived from brains of patients with HIV/AIDS (HIV+) or non-HIV-infected (HIV-) were conducted to detect differentially expressed microRNAs, potentially targeting HIV-1 genes. Based on strong sequence complementation, bioinformatic analyses of selected microRNAs were used to determine possible HIV-1 mRNA target sites within the HIV-1 vpr gene. To evaluate the impact of select miRNAs on Vpr expression, a dual-luciferase reporter assay was created in which several vpr clones with distinct sequence differences were examined. To verify predicted target sites, specific sequences within vpr were mutated. Co-transfections of HEK 293T cells with target clones and select microRNAs were used to determine their effects on vpr expression.

RESULTS

Based on differential microRNA array expression in HIV+ and HIV- brains, we identified multiple miRNAs that potentially targeted HIV-encoded genes, some of which associated with HIV-1 vpr. Bioinformatic analyses disclosed that the microRNAs miR-149 and miR-211 were predicted to target vpr. Preliminary analyses indicate that miR-149, but not miR-211, reduced Vpr protein expression in co-transfected HEK 293T cells.

CONCLUSIONS

These data suggest a potential role for miR-149 in modulating the expression of HIV-1 Vpr and support the capacity of miRNAs to modulate viral gene expression. The ability of disease to perturb microRNA expression profiles may be exploited as a means to monitor disease development and might also lead to new therapeutic strategies.

Supervisor: Dr. Christopher Power

Suppressed angiogenesis drives right ventricular failure.

Paulin R, Sutendra G, Gurtu V, Dromparis P, Stenson T, Haromy A, Bonnet S and Michelakis E.

INTRODUCTION

Right ventricular (RV) failure is the most important prognostic factor for morbidity and mortality in pulmonary hypertension (PHT). Compared to left ventricle (LV), RV is less able to adapt to increased afterload and the transition from a compensated (CRV) to decompensated state (DRV) occurs much earlier. There is some evidence the hypertrophied RV is relatively ischemic. We hypothesized that inappropriate suppression of HIF-1 α signaling inhibits angiogenesis promoting ischemia and transition from CRV to DRV.

METHODS

In a model of monocrotaline induced PHT, we studied free RV wall tissue from rats with normal RV function (NRV, RVSP=38 \pm 2 mmHg, CO=102 \pm 2ml/min, RV/LV+Septum=22 \pm 1), CRV (RVSP=66 \pm 8 mmHg, CO=80 \pm 13ml/min, RV/LV+Septum=48 \pm 3 at 2 weeks post-monocrotaline) and DRV (RVSP=52 \pm 2 mmHg, CO=67 \pm 10 ml/min, RV/LV+Septum=58 \pm 2, ascites, weight loss, 4-6 weeks post monocrotaline) (n=5/group).

RESULTS

Compared to NRV, CRV exhibited increased capillary density but this was reduced in DRV. This decreased angiogenic response in DRV was associated with HIF-1 α inhibition and a reciprocal p53 activation. Moreover, uncoupling protein-2 expression increased in DRV that may explain HIF-1 α inhibition since it was associated with a decrease in the mitochondrial derived pseudohypoxic signals (mitochondrial reactive oxygen species) that can activate HIF1 α even in normoxia. Using Taqman Arrays we studied miRNA expression in NRV, CRV and DRV and found that p53-dependent miR-132, -155, -208a and -92a as well as the HIF-1 α -dependent miR-200b are differentially expressed in CRV and DRV compared to NRV, but also compared to what is known in LV failure. miR-200b, -132 and -208a (which is involved in the regulation of UCP2 expression) levels correlated with RV size (r=-0.68, 0.87, -0.72, respectively) as failure progressed.

CONCLUSIONS

RV failure is characterized by decreased angiogenesis that may be regulated differently than in the LV due to diverse regulation of critical miRNAs, potentially stemming from the fact that the 2 ventricles have different embryologic origin.

Supervisor: Dr. Evangelos Michelakis

Do low stimulated thyroglobulin levels (<2 ug/L) in differentiated thyroid cancer patients after surgery and radioiodine predict recurrent/persistent disease?

M Aldawish, N Jha, AJB McEwan, D Severin, DW Morrish

INTRODUCTION

The stimulated thyroglobulin (Tg) is the most sensitive method to detect residual or recurrent disease in differentiated thyroid cancer (DTC) patients. Multiple studies show that using a recombinant human TSH (rhTSH) Tg level cutoff above 2 ng/mL will identify DTC patients with persistent disease but the subgroup of DTC patients with stimulated Tg levels above lowest laboratory functional sensitivity limit but less than 2 µg/L have not been fully evaluated. Therefore, the purpose of this study was to evaluate this group of patients for recurrence.

METHODS

839 consecutive patients were reviewed and 95 adult DTC patients followed for a median 6 years (range 2-10 years) after total or near-total thyroidectomy and RRA were identified who had stimulated Tg of 0.4-2.0 µg/L at 6-10 months after RRA. The patients were classified as having persistent disease or no evidence of disease (NED) at 10-24 months and final follow-up visit after RRA.

RESULTS

At 10-24 months after RRA, persistent disease was identified in 54 patients (57%). There were 40/54 patients with structural evidence of disease. The remaining 14 patients had biochemical evidence of disease (detectable stimulated or suppressed Tg) without structural evidence of disease. 41 patients (45%) were classified as having NED at 10-24 months after RRA, 27 patients (66%) did not receive further radioactive iodine (RAI) therapy, remained without evidence of disease at a median follow-up of 6.5 years. Stimulated Tg level above 0.6 µg/L at 6-10 months after RRA had sensitivity (83.3%), specificity (56%), positive predictive value (71.5%) and negative predictive value (72%) of detecting persistent disease at 10-24 months after RRA.

CONCLUSIONS

DTC patients at 6-10 months after surgery and RRA with stimulated Tg 0.4-2.0 µg/L need to be evaluated for high potential persistent disease irrespective of initial risk stratification.

Supervisor: Dr. Donald W. Morrish

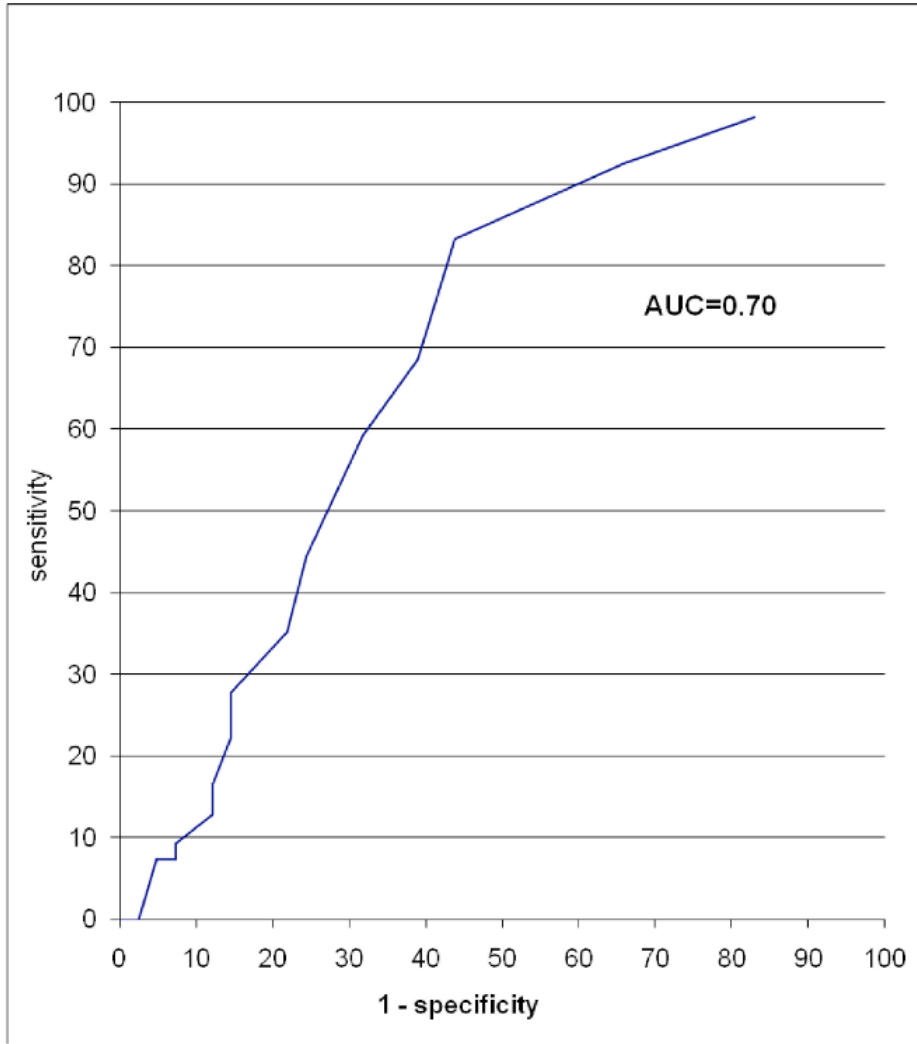


Fig1. Stimulated Tg ROC curve to predict persistent disease at 10-24 months

After RRA.

Association of pre-dialysis care on mortality and renal outcomes in subjects with chronic kidney disease: a propensity score matched case-control population in Alberta

Betty Chui, Scott Klarenbach

INTRODUCTION

Formal pre-dialysis care in chronic kidney disease delivers education, co-ordinated multi-disciplinary care, with purported benefits of delayed end stage renal disease (ESRD) initiation, improved preparation for dialysis initiation and earlier transplantation. The existing evidence on the effectiveness and cost-effectiveness of pre-dialysis care is lacking.

METHODS

Incident patients enrolled in pre-dialysis care in the province of Alberta from 2002 to 2006 were identified and matched to controls through 2:1 propensity score matching based on age, gender, co-morbidities, eGFR, and proteinuria using data from the Alberta Kidney Disease Network. We evaluated the association of pre-dialysis care on mortality, hospitalization, and ESRD care in a fully adjusted logistic regression model.

RESULTS

2113 pre-dialysis care patients were propensity score matched to 4226 control patients. Mortality at 3 years was significantly lower in the pre-dialysis care cohort (odds ratio (OR) 0.82; 95% confidence interval, 0.69-0.98). Transplantation (OR 1.94; 1.32-2.83), dialysis initiation (OR 5.35; 4.42-6.47), and hospitalization use (OR 1.28; 1.10-1.49) were significantly higher in the pre-dialysis care group at 3 years. Of patients who developed ESRD and initiated dialysis at 3 years, patients receiving pre-dialysis care had significantly lower mortality (OR 0.67; 0.47-0.96), were significantly more likely to initiate peritoneal dialysis (OR 3.65; 2.27-5.86), but had no difference in hospitalization (OR 0.76; 0.48-1.20).

CONCLUSIONS

Pre-dialysis care for chronic kidney disease is associated with significantly decreased mortality, increased dialysis initiation, hospitalization use, and increased use of more preferable renal replacement modalities of transplantation and peritoneal dialysis.

Supervisor: Dr. Scott Klarenbach

Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation

Muhammad Asaduzzaman¹, Courtney Davidson¹, Ahmed Nadeem¹, Nancy Arizmendi¹, Lavinia Iuliana Ionescu¹, John Gordon², Morley Hollenberg³, and Harissios Vliagoftis¹

INTRODUCTION

Proteinase-Activated Receptor 2 (PAR2) is activated by serine proteinases and has a pro-inflammatory role in many tissues. We have previously shown that PAR2 activation by serine proteinases in the airways leads to airway hyperresponsiveness (AHR) and inflammation, thus implicating PAR2 in the pathophysiology of asthma. We now hypothesize that functional inhibition of PAR2 during allergen challenge would inhibit allergen-induced AHR and inflammation in a mouse model of asthma.

METHODS

Mice were sensitized intraperitoneally with ovalbumin (OVA) adsorbed to aluminum hydroxide followed by two intranasal (i.n.) challenges with OVA. To investigate the role of PAR2 in the development of AHR and inflammation we administered a blocking anti-PAR2 monoclonal antibody (SAM-11) or an isotype matched control antibody i.n. before both challenges with OVA. A control group received saline only. AHR and airway inflammation were assessed 24h after the last challenge with OVA.

RESULTS

OVA sensitized and challenged mice developed AHR and airway inflammation which was inhibited by administration of the PAR2 blocking antibody. SAM-11 also prevented the development of airway inflammation in response to OVA as seen by lower number of total cells and eosinophils in the BAL fluid and lower levels of cytokines in the lung tissue, but had no effect on OVA-specific serum IgE level. Administration of SAM-11 in vivo also prevented OVA-specific splenocyte proliferation.

CONCLUSIONS

Our results show that administration of an anti-PAR2 antibody during allergen challenge decreases allergen-induced AHR and inflammation in mice. Therefore, functional blocking of PAR2 in the airways may be considered as a potential treatment of allergic asthma.

Supervisor: Dr. Harissios Vliagoftis

Relationships between cardiovagal baroreceptor sensitivity, peak oxygen consumption, and hypoxia in chronic obstructive pulmonary disease (COPD)

Heather Edgell¹, Craig Steinback², Mathew Rieger¹, Norah Vogan¹, Eric Wong¹, Mohit Bhutani¹, and Michael Stickland¹

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) patients typically have both reduced exercise capacity (VO_{2peak}) and reduced cardiovagal baroreflex sensitivity (BRS; Patakas et al., 1982). The underlying mechanism for lower BRS in COPD is unclear. Previous work has shown that lower BRS is associated with aortic stiffness (Mattace-Raso et al., 2007) which in turn is associated with increased cardiovascular risk and mortality (Vlachopoulos et al. 2010). Due to the positive effects of exercise training on both VO_{2peak} and BRS sensitivity, it was hypothesized that lower BRS in COPD would be related to VO_{2peak} . Secondly, other diseases have shown an altered cardiovascular/autonomic response to hypoxia, and therefore we hypothesized that the response of BRS to hypoxia would be attenuated in COPD.

METHODS

COPD patients ($n=14$; Mean $FEV_1=59.4\pm 33.9\%$ predicted, age= 70.9 ± 8.8) and healthy controls ($n=8$; Mean $FEV_1=104.0\pm 12.5\%$ predicted, age= 67.5 ± 5.1) were recruited. None had previously been diagnosed with cardiovascular disease, diabetes or sleep apnea. VO_{2peak} data were obtained from a standard cardiopulmonary exercise stress test. On a separate testing day 5 minutes of uninterrupted resting heart rate and beat-by-beat blood pressure were measured in both normoxic and hypoxic conditions (arterial saturation of oxygen = 85%). BRS was determined using the sequence method.

RESULTS

BRS (7.2 ± 3.8 vs. 14.8 ± 9.7 ms/mmHg; $P=0.01$) and VO_{2peak} (19.6 ± 4.0 vs. 31.1 ± 9.3 mL/kg/min; $P<0.001$) were lower in COPD, and a positive linear relationship in COPD and controls between VO_{2peak} and BRS was observed ($R^2=0.24$; $P=0.02$). BRS was unchanged with hypoxia in both COPD (Normoxia: 7.2 ± 3.8 vs. Hypoxia: 7.6 ± 3.8 ms/mmHg; $P=0.50$) and controls (Normoxia: 14.8 ± 9.7 vs. Hypoxia: 11.5 ± 6.4 ms/mmHg; $P=0.11$).

CONCLUSIONS

Preliminary results indicate that the reduction of BRS is related to reduced exercise capacity in COPD and that there is a trend towards an attenuated response of BRS to hypoxia in COPD; however more statistical power is needed. Funded by Canadian Institutes of Health Research.

Supervisor: Dr. Michael Stickland

Characterization of NT2 neuronal cell line for the study of inflammation-mediated neurodegenerative processes of multiple sclerosis

Haile Y.*, Fu W.*, Shi B.**, Westaway D.**, Baker G.***, Jhamandas J.* and Giuliani F.*

INTRODUCTION

Recent evidence shows that inflammation plays a significant role in mediating neurodegeneration in diseases such as multiple sclerosis (MS). MS is the most common cause of non-traumatic chronic neurological disability affecting young adults. We have shown that inflammatory cells attack neurons and release granzyme-B (GrB), a serine proteases family. GrB diffuses within the neuronal cytoplasm independent of any lytic agent and induces apoptosis; however, the underlying mechanism of GrB-mediated death is unclear. To address this question, we established an in vitro cell culture system using primary human fetal neurons (HFNs) and a more differentiated human neuronal cell-line from human teratoma stem cells (NT2). In the present study we evaluated the maturity and physiological properties of the NT2 cell-line in comparison to the primary HFNs.

METHODS

NT2 cells were cultured on T75 flasks supplemented with DMEM + FBS; and differentiated to neurons by treating with retinoic acid. HFNs were isolated from the brain tissue of fetuses. The maturity and viability of NT2 and HFNs was assessed using immunocytochemistry for MAP-2. Physiological property of HFNs and NT2 cells was evaluated by patch clamping, calcium imaging and HPLC. Parameters such as neurite length, cell size and nuclear area of the cells was analysed by In-cell Western high-throughput imaging.

RESULTS

Our findings showed that treatment of NT2 cells with retinoic acid induce mature neurons. The high-throughput cell imaging showed that NT2-derived neurons have significantly longer neurites, bigger nuclear size, larger area of the whole cell and cell body compared to the primary HFNs. Patch clamping, calcium imaging and HPLC showed that action potential, intracellular calcium fluctuation and amino acid assessments of NT2 cells were similar to HFNs.

CONCLUSIONS

This study shows NT2 neurons are morphologically more differentiated but similar in physiological properties to HFNs. These findings support the use of the readily available NT2 cells as an alternative model to HFNs for the study of inflammatory-mediated neurodegeneration.

Supervisor: Dr. Fabrizio Giuliani

Mesenchymal stromal cells derived from umbilical cord blood migrate in response to complement C1q

Yuanyuan Qiu, Leah A. Marquez-Curtis, and Anna Janowska-Wieczorek

INTRODUCTION

Mesenchymal stromal cells (MSC) have great potential for tissue regeneration and cellular therapy because of their capacity to produce bioactive molecules, differentiate into various tissues, and modulate immune responses. They migrate preferentially to sites of inflammation and tissue injury, but the molecular signals that guide them to their target tissue remain unclear. We previously reported that complement component subcomponent q (C1q), the initiator of classical pathway of complement activation, enhances the homing-related responses of hematopoietic stem/progenitor cells (HSPC). Others reported that complement C3a and C5a chemoattract MSC. Here we investigated whether C1q elicits directional signals that could influence the migration of MSC to injured tissues.

METHODS

Human umbilical cord blood (CB)-derived MSC were maintained for 3-6 passages and characterized for surface marker expression and differentiation potential. Chemoinvasion assay was performed to evaluate the ability of MSC to cross the reconstituted basement membrane Matrigel and Gelatin. RT-PCR, flow cytometry, Western blot and zymography were used to determine the gene and protein expression of C1q receptors, secretion of matrix metalloproteinases (MMPs), and phosphorylation of extracellular signal-regulated kinase (ERK).

RESULTS

We found that C1q chemoattracted MSC in a dose-dependent manner, and the receptor for the global domains of C1q (gC1qR) is present on the surface of MSC. Further, C1q enhanced/primed the homing-related response of MSC towards a low gradient of stromal cell-derived factor-1 (SDF-1), known to be upregulated at sites of injury, partly due to an increase of the SDF-1 receptor CXCR4. Moreover, C1q increased the secretion of MMP-2 and induced phosphorylation of ERK1/2.

CONCLUSIONS

This study indicates that C1q mediates the migration of MSC in two ways: first, by acting as a chemoattractant, and second, by priming chemotactic response to SDF-1. Our findings suggest new molecular mechanisms of MSC migration that may contribute to their clinical application in tissue repair.

Supervisor: Dr. Anna Janowska-Wieczorek

LENTIVIRUS NEUROVIRULENCE IS VIRAL STRAIN-DEPENDENT: REGULATION OF TETHERIN/CD317 IN BRAIN

Polyak MJ, Vivithanaporn P, Maingat F, Pearson K, Meeker RB, Cohen EA, Power C

INTRODUCTION

Infections of the nervous system by lentiviruses including human (HIV-1) and feline (FIV) immunodeficiency viruses often cause neurological disease, termed neurovirulence. Variable severity in neurovirulence has been reported in lentivirus models as well as in humans infected by different viral strains or clades. The interferon-responsive gene, Tetherin/CD317, has been implicated in HIV/AIDS pathogenesis as a host restriction factor, although its expression and function in the nervous system is unknown. Herein, we examined the expression and actions of Tetherin in the setting of lentivirus neurovirulence.

METHODS

Host gene and protein expression was measured in HIV-1- and FIV-infected and uninfected brains and cultured neural and immune cells by real time RT-PCR, immunohistochemistry and western blotting. Animals infected with one of two strains of FIV, FIVch or FIVncsu, were compared to mock-infected animals in terms of neurobehavioral, molecular and neuropathological aspects.

RESULTS

Tetherin was constitutively expressed in cultured human microglia together with showing enhanced expression in brain myeloid cells among HIV-infected individuals. Moreover, Tetherin was induced in microglia by interferon alpha exposure and HIV-1 infection. Viral strain-dependent Tetherin induction was apparent for both HIV-1 and FIV infections of leukocytes. While FIVch and FIVncsu in vivo infections caused similar levels of CD4 T cell suppression and viral burden, the neuroinflammatory genes CD3 epsilon and TNF alpha were increased in brains from FIVch-infected animals. In contrast, Tetherin and Mx1 were increased in the brains of FIVncsu animals. These molecular findings were associated with increased neurobehavioral deficits and cortical neuronal loss among FIVch-infected animals as compared to mock- and FIVncsu-infected animals.

CONCLUSIONS

The present studies indicate that lentivirus strain is an important determinant of neurovirulence which is associated with altered expression of the immunomodulatory molecule Tetherin. Induction of Tetherin in myeloid cells might represent an immune regulatory pathway which limits neurovirulence.

Supervisor: Dr. Christopher Power

“Histamine: a new therapeutic target for Alzheimer’s disease”.

Patel AN, Vasanthan V, Fu W, Jhamandas JH

INTRODUCTION

Beta-amyloid (A β) accumulation and neuritic plaque formation in the brain are major neuropathological hallmarks of Alzheimer’s disease (AD). One of the strategies proposed to alter the A β deposition in the brain is promotion of A β catabolism. Matrix Metalloproteinase-9 (MMP-9), a member of the family of Zn⁺² containing endoproteases, known to be expressed and secreted by astrocytes, has been shown to effectively degrade A β . Interestingly, histamine, a major aminergic neurotransmitter in the brain, has been shown to stimulate the production of MMP-9 in keratinocytes through histamine H₁ receptor. Based on these observations, we hypothesized that histamine-evoked increase in MMP-9 release from astrocytes may promote potentially beneficial clearance of A β deposits in the brain.

METHODS

Primary cortical astrocytic cultures and a human U373 astrocytic cell line were established and exposed to different concentrations of histamine (750 nM – 100 μ M) and also histamine antagonists (H₁, H₂, H₃ and H₄ receptor) (5 – 75 μ M) for varying lengths of time. Standard gelatine zymography and Ca⁺² imaging were used in this study.

RESULTS

Histamine increased the MMP-9 release in a concentration- and time- dependent manner. H₁ receptor (R) but not H₂, H₃ and H₄ R antagonists blocks histamine induced increased MMP-9 release. Histamine induces concentration-dependent elevations in intracellular Ca⁺². H₁ R but not H₂ and H₃ R antagonists, blocked histamine induced Ca⁺² responses in astrocytic cells. Moreover, histamine induced MMP-9 release was completely blocked in Ca⁺² free culture media compared to normal media.

CONCLUSIONS

Histamine release of MMP-9 is mediated via histamine H₁ R subtype. Moreover, histamine, through H₁ R mediated effect, evokes an increase in intracellular Ca⁺². Additionally, histamine induced increase in MMP-9 release is Ca⁺² dependent. MMP-9 release may catabolize A β and provide neuroprotection in AD. Histamine may therefore serve as a useful therapeutic target to treat AD.

Supervisor: Dr. Jack H. Jhamandas

Pharmacist Intervention for Glycemic Control in The Community (The RxING Study); Baseline Characteristics and Study Status

Al Hamarneh YN (1), Charrois TL (1,2), Lewanczuk RZ (1), Tsuyuki RT (1)

INTRODUCTION

Approximately 2 million Canadians are living with diabetes and this figure is expected to rise by 75% by 2030. Pharmacists are front line healthcare professionals who see patients with diabetes frequently, and in Alberta they have been granted authority to prescribe medications. As such, they could systematically identify poorly controlled patients and help improve their glycemic control. The aim of this study is to determine the effect of a community pharmacist prescribing intervention on glycemic control.

METHODS

Study design: Pragmatic, before-after design

Setting: 10 community pharmacies in Alberta.

Patients: Type 2 diabetes patients receiving oral hypoglycemic medications and with HbA1c of 7.5-11%

Recruitment: Pharmacists systematically identify potential candidates by inviting patients with Type 2 diabetes to test their HbA1c using validated point of care technology (DCA Vantage®)

Intervention and Follow-up: Pharmacists prescribe 10 units of insulin glargine at bedtime, adjusted by increments of 1 unit daily to achieve a morning fasting glucose of ≤ 5.5 mmol/L. The patients are followed at 2, 4, 8, 14, 20, and 26 weeks. The primary outcome is the change in HbA1c from baseline to week 26.

RESULTS

Recruitment: We screened 303 patients of which 84 were eligible. Of these, 73 (87%) have been enrolled in the study; all 11 patients who did not consent because of refusal to use insulin.

Baseline Characteristics: Average age is 64 years (standard deviation (SD) 10.68), 53% are male, 87% have a BMI > 25 kg/cm² and 79% have elevated waist circumference. Average diabetes duration is 10.14 years (SD 7.11), and the most widely prescribed oral medication is metformin (86%). Average HbA1c is 8.86% (SD 0.94) and average fasting blood glucose is 10.98 mmol/L (SD 4.25).

CONCLUSIONS

This ongoing study will provide evidence for pharmacists' ability to improve glycemic control and improve access to care. Recruitment of the target of 100 patients is expected by March with the results available by October 2012.

Supervisor: Dr. Ross Tsuyuki

Dietary intake and development of a population specific food frequency questionnaire for rural South Africans

Mathe, N., Kolahdooz, F., Sheehy, T., Spearing, K.P., Lukasewich, M., Bain, K.E., Mtshali, T.L., Ngubane, X., Sharma, S

INTRODUCTION

Food availability, accessibility and preferences vary between settings, ethnic groups and culturally distinct populations, it is important to develop dietary assessment tools that are specific to the population. We aimed to assess the diets of adults in rural KwaZulu-Natal and to develop a culturally specific quantitative food frequency questionnaire (QFFQ).

METHODS

Cross-sectional study assessing diet in 81 adults. One 24-hour dietary recall was collected from each individual. A draft-QFFQ was developed from foods reported in the 24-hour recalls by more than one subject, except for foods contributing little caloric value (e.g. condiments); in addition culturally appropriate portion sizes were selected.

RESULTS

Results are presented for men and women in two age groups (19-50 and >50 years). Women had significantly higher intakes of energy compared to men in both age groups, although there were no significant differences in percentage of energy from individual macronutrients (carbohydrate, protein, fat) between men and women of either age group. Median percentage of energy from fat was below the recommendation (20-35%) for all participants, whereas median percentage of energy from protein was within the recommended range (10-35%), albeit at the lower end (13-16%). For all age groups, median intakes of micronutrients in particular vitamin B5, B12, C, D, E and calcium were below the Dietary Reference Intake (DRI). Among participants aged 19-50 years, men had significantly lower intake of vitamin E compared with women ($p < 0.05$). Older men had significantly lower intakes of iron, selenium and zinc compared with women (all $p < 0.05$). The developed QFFQ included 71 food items; 16 were composite dishes unique to this population.

CONCLUSIONS

Overall, the diet was monotonous characterized by a high intake of energy from carbohydrates, and low intakes of micronutrients. The QFFQ will allow for assessment of diet and identification of the extent of the nutrient adequacy in this population.

Supervisor: Dr. Sangita Sharma

Defining abnormal metabolism in Primary Biliary Cirrhosis.

Bo Meng, Weiwei Wang, Siqi Liu, Andrew Mason

INTRODUCTION

Primary biliary cirrhosis (PBC) is associated with the formation of anti-mitochondrial antibodies (AMA). Previous studies of PBC biliary epithelium have shown increased numbers of swollen mitochondria with abnormal phenotype as well as aberrant location of PDC-E2 on the cell surface. To better understand it, we have studied the transcriptional and metabolic changes in BEC from patients with PBC and control subjects. To date we have found evidence of activation of glycolysis and oxidative phosphorylation in PBC BEC. We hypothesize that PBC BEC have a unique metabolic phenotype associated with abnormal distribution of PDC-E2 in cells.

METHODS

Four liver samples from PBC patients, PSC patients and cryptogenic cirrhosis patients were labelled by different CyDye DIGE Fluor and assessed by 2D gel analysis. Approximately 1900 spots were detected on each gel and differentially expressed proteins were identified and characterized using MALDI-TOF/TOF.

RESULTS

to date, 46 proteins were identified. Compared to PSC, PBC patients had increased expression of two proteins associated with glycolysis (PEPCK [GTP], ADH4) as well as 4 isoforms of ATP synthase, a component of the F1 complex in mitochondria, which are consistent with previous metabolic study of PBC BECs and support our hypothesis. Moreover, two proteins associated with amino acid biogenesis (BHMT isoform CRA_b and TGM6) were increased in PBC liver; these data are also consistent with the glycolytic phenotype found in cancers where the glycolytic metabolites are used for generating amino acids and proteins. Altered ANXA2 expression was observed in PBC liver that directly impacts on AE activity in cholangiocytes, which is known to have a role in the pathogenesis of PBC. A protein kinase TRRAP involved in HTLV-1 infection process was found to be up-regulated in PBC liver.

CONCLUSIONS

our results unveil several proteins implicated in the pathogenesis of PBC that require further validation and investigation.

Supervisor: Dr. Andrew Mason

Loss of ACE2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the Ang II/AT1 receptor axis

Vaibhav B. Patel 1,2, Sreedhar Bodiga 1,2, Ratnadeep Basu 2,3, Subhash Das1,2, Wang Wang 2,3, Zuo Cheng Wang 1,2, Jennifer Lo 1,2, Maria B. Grant 4, JiuChang Zhong 1,2, Zamaneh Kassiri 2,3 and Gavin Y. Oudit 1,2,3

INTRODUCTION

Diabetic cardiovascular complications are reaching epidemic proportions. ACE2 is a negative regulator of the renin-angiotensin system. We hypothesize that loss of ACE2 exacerbates cardiovascular complications induced by diabetes. Objective of the present study was to define the role of ACE2 in diabetic cardiovascular complications.

METHODS

We used the well-validated Akita mice, a model of human diabetes, and generated double mutant mice using the ACE2 knockout (KO) mice (Akita/ACE2^{-/-}).

RESULTS

Diabetic state was associated with increased ACE2 in Akita mice, while additional loss of ACE2 in these mice leads to increased plasma and tissue Ang II levels resulting in systolic dysfunction on a background of impaired diastolic function. Downregulation of SERCA2 and lipotoxicity were equivalent in Akita and Akita/ACE2KO hearts and are likely mediators of the diastolic dysfunction. However, greater activation of protein kinase C and loss of Akt and eNOS phosphorylation occurred in the Akita/ACE2KO hearts. Systolic dysfunction in Akita/ACE2KO mice was linked to enhanced activation of NADPH oxidase and metalloproteinases resulting in greater oxidative stress and degradation of the extracellular matrix. Impaired flow-mediated dilation *in vivo* correlated with increased vascular oxidative stress in Akita/ACE2KO mice. Treatment with the AT1 receptor blocker, irbesartan rescued the systolic dysfunction, normalized altered signaling pathways, flow-mediated dilation and the increased oxidative stress in the cardiovascular system.

CONCLUSIONS

Loss of ACE2 disrupts the balance of the RAS in a diabetic state and leads to an Ang II/AT1 receptor-dependent systolic dysfunction and impaired vascular function. Our study demonstrates that ACE2 serves as a protective mechanism against diabetes-induced cardiovascular complications.

Supervisor: Dr. Gavin Y. Oudit

Crohn's Disease Genotype Is Similar Between Patients Who After Discontinuing Infliximab Sustain A Long-Term Remission Versus Those Who Rapidly Relapse

C. Lu, A.W. Waugh, R. Bailey, R. Cherry, L. Dieleman, L. Gramlich, K. Matic, M. Millan, K. Kroeker, D.C. Sadowski, C.W. Teshima, D. Todoruk, C. Wong, K. Wong, R.N. Fedorak

INTRODUCTION

The majority of patients with Crohn's disease who experience an infliximab-induced corticosteroid-free clinical remission will relapse once infliximab therapy has been discontinued; some of these patients relapse rapidly and others enter a sustained clinical remission. Nevertheless, to date there are no phenotypic markers that predict the duration of remission after infliximab is discontinued, implying a genetic difference may be responsible. This study was the first to compare genetic differences (IBD5, NOD2/CARD 15) between Crohn's disease patients who after discontinuing their infliximab while in full corticosteroid-free clinical remission, experienced a sustained remission versus those patients whose disease relapsed rapidly.

METHODS

Genetic analyses were performed on samples obtained from 14 Crohn's disease patients (n=6 who had a sustained long term remission after stopping infliximab, n= 8 who rapidly relapsed after stopping infliximab). All of these patients had experienced full corticosteroid-free clinical remission and had later discontinued infliximab for reasons other than loss of response.

RESULTS

There was no significant increase in frequency of the NOD2/CARD15 polymorphisms (R702W, G908R and L1007fs) and the IBD5 polymorphisms (IGR2060a1 and IGR3081a1) in either group of patients; those whose disease relapsed rapidly or those who remained in sustained long term remission following the discontinuation of infliximab. Interestingly, the patients who lost remission did so after 1.0 ± 0.6 years, while those still in remission were at the time of this study, 8.1 ± 2.6 years post-discontinuation of infliximab, $p < 0.001$.

CONCLUSIONS

Nearly a third of patients in full clinical remission who stop infliximab for reasons other than loss of response remain in sustained clinical remission, while two-thirds relapse rapidly. There are no IBD5 or NOD2/CARD15 mutations that predict which patients might have sustained remission and which will relapse rapidly after stopping infliximab.

Supervisor: Dr. Richard Fedorak

The Impact of Chronic Obstructive Pulmonary Disease on the clinical features of patients with co-existing Heart Failure

Jason Weatherald MD, Justin Ezekowitz MD, Eric Wong MD, Michael Stickland MD, Mohit Bhutani MD

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) and Heart Failure (HF) commonly coexist. Little is known about the impact of COPD on clinical features or outcomes of patients with HF.

METHODS

We conducted a retrospective study of 105 patients with known HF, both systolic and diastolic, who are enrolled in Alberta HEART, an observational cohort. Patients were divided into one of three groups: (1) known COPD; (2) no COPD but a history of smoking; and (3) no COPD and non-smokers. Groups were compared on a variety of clinical features and outcomes.

RESULTS

21% of patients had an existing diagnosis of COPD. 37% had a smoking history without a diagnosis of COPD. Comorbid conditions were similar between the three groups, however, the diagnosis of obstructive sleep apnea was more common in the COPD group (50.0% vs 15.4% vs 11.4%, $p=0.001$). Patients in group 1 were less likely to be prescribed carvedilol than patients in Group 2 and 3 (4.5% vs 34.9%). Groups 1 and 2 were more likely to have a preserved ejection fraction HF (LVEF > 45%) than patients in group 3 (63.6%, 61.5% vs 37.2%, $p=0.04$). Severe HF symptoms (NYHA III or IV) were more frequent in patients with COPD than those without COPD (50.0% vs 25.3%, $p=0.03$). Logistic regression analysis showed that the diagnosis of COPD predicted the presence of NYHA III or IV symptoms (adjusted OR 4.40, 95% CI 1.07 – 18.09, $p=0.04$). COPD inhaler therapy was appropriate in only 50% of COPD patients.

CONCLUSIONS

In patients with HF, a co-existing diagnosis of COPD is associated with a more severe functional class, preserved LVEF and altered cardiac medical management. Gaps in COPD medical treatment were also identified.

Supervisor: Dr. Mohit Bhutani

Crohn's Disease Patients Treated With Infliximab Have Normal Pulmonary Function With Complete Remission But Diminished Pulmonary Function With Partial Response

Dr. Farhad Peerani, Dr. Brendan Halloran, May Choi, Dr. Justin Weinkauff, Dr. Richard Fedorak

INTRODUCTION

Pulmonary involvement is a recognized complication of Crohn's disease (CD); however, its clinical impact is not well understood. It is unclear how treatment of CD affects disordered pulmonary function or whether residual disease activity during CD treatment results in ongoing pulmonary dysfunction. The purpose of this study was to examine pulmonary function in a population of CD patients on infliximab by comparing patients with ongoing clinical disease activity to patients in complete clinical remission.

METHODS

CD patients on infliximab who had demonstrated a response to or remission to infliximab were enrolled between May and July 2011. Patients completed a questionnaire detailing CD activity and their pulmonary history including: smoking, the MRC dyspnea index and respiratory symptom score. CD activity was assessed via the Harvey Bradshaw Index (HBI); active disease defined as HBI ≥ 5 and remission < 5 . Each patient underwent a CXR, PFT, 6-minute walk test and screening blood testing. One blinded physician interpreted all pulmonary investigations. Data was reported as mean \pm standard error of the mean and analyzed using Wilcoxon Mann Whitney nonparametric tests.

RESULTS

63 sequential patients (25 active, 38 remission) were recruited. Differences between the active CD group and remission CD group, respectively, included MRC dyspnea index (1.6 ± 0.1 , 1.2 ± 0.1), presence of respiratory symptoms (24%, 5.3%), and partially reversible airway obstruction (32%, 7.9%) [$p < 0.05$]. There was no significant difference between the groups with respect to smoking history or current steroid use. Furthermore, diffusing capacity and small airways obstruction was similar in both groups. Active and inactive CD showed equivalent values in their 6-minute walk tests.

CONCLUSIONS

This study demonstrates that in a cohort of CD patients who have responded to infliximab, those with ongoing disease activity display more pulmonary symptoms, a higher dyspnea index and more partially reversible airway obstruction when compared to patients with CD in full remission.

Supervisor: Dr. Richard Fedorak

Edmonton Rheumatology Triage System: Review of Initial Implementation and Effect on Wait Times for Inflammatory Arthritis

Thirza Carpenter and Steven J. Katz

INTRODUCTION

The Division of Rheumatology at the University of Alberta recently created a triage system to ensure timely access to care. We review 20-month data, including examination of access to care for inflammatory arthritis patients prior and subsequent to the introduction of the triage system.

METHODS

The triage rheumatologist, typically using only the information provided in the referring letter and any included investigations, screens all incoming referral letters to identify possible diagnoses and urgency of assessment. After the initial patient visit, the consulting rheumatologist records a post-visit diagnosis and if they agree with the assigned urgency status. The system was devised so patients triaged as "soon", such as those with possible inflammatory arthritis, could be seen within 6 weeks. Triaged patients' wait-times, defined as from time of referral to clinic visit, and pre and post-triage diagnosis were compiled in a database. We report this descriptive data from the triage process, emphasizing the inflammatory arthritis group. The wait-time for inflammatory arthritis was also compared to a random sample of inflammatory arthritis patients from the year preceding the triage system implementation.

RESULTS

A total of 3476 new referrals were seen, with an overall average wait-time of 60.0 days. 2183 referrals were triaged as "routine", with an average wait-time of 94.7 days; 1137 as "soon", with an average wait of 31.4 days; 131 referrals as "urgent" with an average wait of 8.7 days. Of the new referrals, 343 patients had a final diagnosis of inflammatory arthritis with an average wait-time of 58.9 days; 189 were appropriately assigned "soon" with an average wait-time of 31.4 days. The majority of those with an inaccurate urgency status were due to miss-assigned urgency status associated with learning the new triage system, not misdiagnosis. In the year prior to the triage system, the inflammatory arthritis sample group (N=49) had an average wait-time of 66.0 days. When comparing the two inflammatory arthritis groups, the overall average wait-time was not significantly reduced ($p=0.1452$), but the average wait-time for inflammatory arthritis appropriately triaged as "soon" was reduced by 34.6 days ($p=<0.0001$).

CONCLUSIONS

This triage system effectively reduces wait-times for targeted patient groups that require more urgent care, provided they are identified correctly in the triage process. Utilization of a triage system may be universally applicable and effective way to ensure appropriate patient care.

Supervisor: Dr. Steven J. Katz

Characteristics	LOS (Days) in Hypnotics	LOS (Days) in Non-Hypnotics	P Value
Age<70	9.33 ± 6.20	6.30 ± 4.41	0.006
Age>=70	11.32 ± 7.39	7.58 ± 6.26	0.004
Male	9.72 ± 6.66	6.40 ± 4.73	0.004
Female	10.89 ± 7.08	7.79 ± 6.39	0.017
Mild (Gold 1)	-	-	-
Moderate (Gold 2)	11.09 ± 5.90	7.60 ± 5.18	0.083
Severe (Gold 3)	10.47 ± 6.03	6.94 ± 5.74	0.036
Very Severe (Gold 4)	12.33 ± 8.35	6.66 ± 2.96	0.003

Edmonton Rheumatology Triage System: Etiological Review of Inaccurate Triage for Inflammatory Arthritis Patients

Thirza Carpenter and Steven J. Katz

INTRODUCTION

We review the accuracy of the rheumatology triage system at the University of Alberta for correctly identifying inflammatory arthritis patients within the triage process and to search for factors that may improve the system accuracy.

METHODS

The triage rheumatologist, typically using only the information provided in the referring letter and any included investigations, screens all incoming referral letters to identify possible diagnoses and urgency of assessment. After the initial patient visit, the consulting rheumatologist records a post-visit diagnosis and if they agree with the assigned urgency status. The system was devised so patients triaged as “soon”, such as those with possible inflammatory arthritis, could be seen within 6 weeks. We reviewed a 20-month database to identify all newly referred inflammatory arthritis patients who were incorrectly screened at the time of triage as a non-inflammatory process. We reviewed these charts to identify patient characteristics that may have negatively influenced appropriate triage, including ESR, CRP, RF, anti-CCP and imaging studies, as well as presence of morning stiffness, joint swelling and location of joint involvement included in the referral letter.

RESULTS

Since implementation of the triage system, 343 newly referred patients were diagnosed with inflammatory arthritis. 31 patients (9.0%) were incorrectly screened as a non-inflammatory process. The average age was 51.2 years and 23 patients were female (71.9%). 24 patients (75.0%) had one or more inflammatory markers available, with only 3 patients (9.4%) having an abnormally elevated value. 2 patients (6.3%) were RF positive, and 1 was anti-CCP-antibody positive from testing performed post-initial visit. Imaging changes consistent with an inflammatory arthritis were seen in 4 patients (12.5%). 4 patients' referral letters specifically included ‘morning stiffness’ or ‘swollen joints’ in a typical inflammatory distribution, while the remaining referrals (84.4%) did not comment on joint stiffness or swelling, specify the swelling distribution, or described an atypical distribution for synovitis. In total, these findings represent 10 patients.

CONCLUSIONS

The triage system correctly identified patients with inflammatory arthritis with an accuracy of 91.0%. This system appears cost-effective because it does not require any specific screening investigations to be performed or reviewed prior to assessment. In fact, it remains unclear if investigations would improve the system accuracy.

Supervisor: Dr. Steven J. Katz

Development of a Factor VIII Inhibitor in Association with Giant Cell Arteritis: A Case Report

Thirza Carpenter and Steven J. Katz

INTRODUCTION

We describe the case of an unusual presentation of acquired hemophilia, an uncommon autoimmune disorder caused by the production of autoantibodies against coagulation factor VIII. While most often idiopathic, it may present as part of an underlying systemic disorder, including many rheumatologic disorders, rarely giant cell arteritis (GCA).

METHODS

A 56 year old woman initially presented with recurrent headaches, jaw claudication and tender, palpable temporal arteries. Inflammatory markers were elevated (ESR 89, CRP 137.9); GCA was confirmed on temporal artery biopsy. She was started on high-dose prednisone with resolution of her symptoms. However, 6 months into her prednisone taper, while on a dose of 9-mg daily, she re-presented with widespread, spontaneous bruising. On further evaluation, she was found to have extensive ecchymosis on her extremities; diagnostic imaging revealed a rectus sheath hematoma. Investigations revealed a normal platelet count and INR with an elevated PTT of 84. A mixing study was performed which showed failure of the PTT to correct, suggesting the presence of a factor inhibitor. Acquired hemophilia was confirmed with a low Factor VIII level of 0.08 and an elevated FVIII inhibitor level of 20.6. Immune suppressive therapy was initiated with high dose prednisone, oral cyclophosphamide and IVIG. No bleeding episodes requiring the use of recombinant factor VIII or anti-inhibitor coagulant complex occurred. Prior to her presentation and throughout the course of her illness, her GCA remained asymptomatic; she did not have recurrence of her headache or visual symptoms. Repeat inflammatory markers (ESR 36, CRP 11.4) were minimally elevated at the time of her presentation.

RESULTS

This case is an extremely unusual presentation of an extremely rare disease. It may be idiopathic, approximately 50%, or present as part of an underlying systemic disorder, including many rheumatologic disorders or malignancy. This is the first reported case of an association between an apparently well-controlled underlying vasculitis and acquired hemophilia. When associated with rheumatologic disorders, previous reports have only documented active or undiagnosed conditions.

CONCLUSIONS

Acquired hemophilia has a known association with undiagnosed systemic diseases and malignancies. We demonstrate that rheumatic diseases, such as giant cell arteritis, can now be shown to cause production of anti-factor VIII antibodies, even when appropriately treated and seemingly quiescent. Acquired hemophilia should therefore remain on the differential diagnosis of patients with well-controlled rheumatologic disorders who present with spontaneous bruising or bleeding.

Supervisor: Dr. Steven J. Katz

RETROSPECTIVE ANALYSIS OF OUTCOMES OF YOUNG PATIENTS (AGE<40 YEARS) WITH COLORECTAL CANCER (CRC) IN NORTHERN ALBERTA

Toor E, Spratlin J, Mulder K, Ghosh S

INTRODUCTION

Colorectal cancer (CRC) is rare in patients (pts) < 40. Controversies exist in medical literature regarding risk factors and prognosis in this group. We evaluated the risk factors for CRC and the observed benefits from chemotherapy including disease free survival (DFS), overall survival (OS), and progression free survival (PFS).

METHODS

A retrospective chart review was conducted on pts < 40 diagnosed with CRC from January 1, 2000 to December 31, 2010 at the Cross Cancer Institute (CCI). Data collection included: age, gender, presenting symptoms, risk factors (family history of CRC, known genetic predisposition, inflammatory bowel disease), site of primary lesion, stage at diagnosis, histological risk features (grade, lymphatic, perineural or vascular invasion) and treatment (surgery, radiation and chemotherapy). For metastatic CRC (mCRC) pts, OS and PFS analysis was done.

RESULTS

A total of 123 pts met inclusion criteria, of which 65 had mCRC. The rectum and rectosigmoid region were the most common primary sites of disease (48.78%). Bleeding per rectum and change in bowel habits were identified as the most common presenting symptoms. The majority of the pts did not have any identifiable risk factors (78.4%). PFS for mCRC pts receiving 1, 2, 3, and 4 lines of chemotherapy were 9.03, 3.74, 3.9 and 3.31 months respectively. OS for the mCRC group was 18.36 months.

CONCLUSIONS

Despite the similarities in PFS compared to historical controls with 1-4 lines of chemotherapy, OS in patients < 40 treated at the CCI is worse than what is expected based on contemporary clinical trial data. DFS and OS in pts with stage II and III CRC and a breakdown of PFS and OS in mCRC pts treated with varying chemotherapeutic regimens will be presented.

Supervisor: Dr. Karen Mulder/Dr. Jennifer Spratlin

Vitamin D and IBD: A systematic Review

Dr. Noreen Rajwani

INTRODUCTION

Inflammatory bowel disease (IBD) encompasses Ulcerative Colitis (UC) and Crohn's Disease (CD) and is characterized by chronic idiopathic intestinal inflammation. This inflammation is primarily mediated by inappropriate production of pro-inflammatory cytokines by CD4+ T effector cells. Amongst the CD4+ T effector cells regulators, Vitamin D (25-hydroxyvitamin D) levels have been shown to have positive immune-modulating effects in IBD patients. This has a profound significance for the North American population where disease states such as IBD and Vitamin D deficiency are more prevalent.

The purpose of our study is to conduct a systematic review of prospective studies assessing the association of serum levels of Vitamin D with disease severity in Inflammatory Bowel Disease patients.

METHODS

Relevant studies prior to January 2012 were identified by a search of MEDLINE, COCHRANE and EMBASE databases. The MeSH terms "Vitamin D" and "Inflammatory Bowel Disease" or "Crohns disease and Ulcerative Colitis" were used to identify relevant studies. These were further limited to English language, studies performed in adults and work completed after 1995. The quality of the study was then evaluated with numerical scoring criteria and inclusion restricted to prospective studies above a minimum cutoff.

RESULTS

Clinical endpoints such as disease severity and relapse rates will be correlated with serum Vitamin D levels. Our preliminary hypothesis is that serum Vitamin D levels are inversely proportional to the disease severity and frequency of relapse in Inflammatory Bowel Disease patients.

CONCLUSIONS

This preliminary review will be followed by a retrospective chart analysis of a database composed of IBD patients with various lab parameters measured throughout the course of illness. Ultimately, this will lead to recruitment of patients for a prospective study with design informed by the findings from preliminary work. The clinical implications are that early recognition, screening and management of Vitamin D deficiency may decrease the severity and frequency of IBD.

Supervisor: Dr. Richard Fedorak

Mechanical Circulatory Support for Cyclophosphamide Induced Cardiomyopathy: A case report and review

Sumandeep Dhesi*, MD; Michael Chu*, MD; Gregg Blevins†, MD, FRCPC; Ian Paterson*, MD, FRCPC; Loree Larratt‡, MD, FRCPC; Gavin Y Oudit*, MD, PhD, FRCPC; Daniel H Kim*, MD, FRCPC

INTRODUCTION

CYC is increasingly used for various refractory autoimmune conditions. Fatal cardiac toxicity has been reported in several case reports and series.

METHODS

We report the case of a 28 year old female who develops severe toxic myocarditis after initiation of CYC for refractory neuromyelitis optica spectrum disorder. Our patient was successfully bridged to mechanical circulatory support but died of complications related to ischemic colitis. We also review the literature pertaining to this important clinical entity and propose some diagnostic and management strategies.

RESULTS

(combined as methods and results above)

CONCLUSIONS

Early recognition of CYC-induced cardiac toxicity and initiation of circulatory support, including mechanical circulatory support, may be potentially life-saving.

Supervisor: Dr. Daniel H Kim

Pixel Intensity: A Novel Method of Quantifying Ejection Fraction in 3D Contrast-Enhanced Echocardiography

Xiangning Fan, Jonathan Choy and Harald Becher

INTRODUCTION

Quantifying ejection fraction is of great importance to clinical medicine. Echocardiography is widely utilized to assess cardiac function, and can be performed in two or three dimensions. Intravenous contrast agents can be employed to improve visualization to the human eye, but commercially available software cannot accurately determine ejection fraction in three dimensional (3D) contrast-enhanced data sets. Our goal was to develop a computer-assisted method to assess ejection fraction in 3D contrast-enhanced datasets.

METHODS

Multiple 3D contrast-enhanced datasets from six patients undergoing clinically indicated echocardiography at the Mazankowski Heart Institute in January 2012 were acquired with a Philips ATL iE33 ultrasound machine (Philips, Andover, Mass). Philips QLab quantification software was used to obtain a stack of short axis slices of the left ventricle for each dataset. The myocardium was outlined in short axis on each slice and the cross-sectional areas integrated across the length of the ventricle in systole and diastole to estimate ejection fraction. Simultaneously, for end-systole and end-diastole, pixel intensity within an area of interest on each slice was used to calculate ejection fraction, after accounting for background pixels. Image processing and pixel intensity determination were performed using Matlab (Mathworks Inc.).

RESULTS

Good correlation was demonstrated between ejection fraction derived from change in cross-sectional area and ejection fraction derived from change in pixel intensity (R^2 0.92). Change in pixel intensity on short axis slices tended to overestimate compared to direct measurement of changes in cross sectional area in the same slices (mean difference 5.74 absolute percentage points, standard deviation 4.86).

CONCLUSIONS

Change in pixel intensity over a region of interest correlates well with direct measurement of change in cross sectional area over a stack of short axis slices through the left ventricle. This new method of estimating ejection fraction does not require precisely delineating the myocardial boundary.

Supervisor: Dr. Jonathan Choy/Dr. Harald Becher

ATTITUDES OF PATIENTS ABOUT BEING SEEN BY MEDICAL STUDENTS IN A CANADIAN DERMATOLOGY CLINIC

Isaiah Day, MD; Gian Jhangri, MSc; Andrew N Lin, MD, FRCPC

INTRODUCTION

In academic clinics, patients are often seen by medical students, who then present their findings to faculty dermatologists. This study identifies attitudes of patients concerning these encounters.

METHODS

Consecutive patients 18 years or older who were seen at a general academic dermatology clinic in Canada were asked to complete a questionnaire, requesting demographic data, and attitudes concerning specific aspects of the encounter.

RESULTS

Patients were 62.4% male, 82.4% Caucasian, and 76.4% had more than a high school education. Their age distribution was: 18-30 years (19.5%), 31-40 (11.0%), 41-60 (36.0%), and 61+ (34.0%). The majority felt comfortable, based on patient's age, gender, ethnicity, education, seeing students (85.3%-95.7%), and having the student perform procedures such as skin biopsy with the dermatologist present (84.6%-100%), but only 41.4%-63.0% felt comfortable without the dermatologist present. 50% of patients correctly identified medical students as "a student who is trying to get a medical degree". Patients aged 18-30 and 31-40 years compared to older patients (41-60 and 61+ years) are more likely to want students of the same gender (28.2%, 31.8%, 7.1%, 13.6%, $p=0.006$). Non-Caucasian patients compared with Caucasian patients are more likely to want students of the same gender (31.4% vs. 13.0%, $p=0.008$), and slightly less felt comfortable seeing students (85.3% vs. 95.1%, $p=0.035$). Also, patients with high school or less compared to those with more than high school education are more likely to want students of the same gender, but this was not statistically significant (23.4% vs. 14.1%, $p=0.132$).

CONCLUSIONS

Younger patients (age 18-40) and non-Caucasian patients are more likely to want to be seen by students of the same gender ($p=0.006$ and 0.008 , respectively), and slightly less percentages of non-Caucasian compare to Caucasian patients feel comfortable seeing student (85.3 vs. 95.1, $p=0.035$). Attention to these attitudes will likely improve patient satisfaction and medical student education.

Supervisor: Dr. Andrew Lin

PROBABLE OVER-REPRESENTATION OF ASIAN PATIENTS WITH ISCHEMIC COLITIS

C. Evaschesen, B. Mangat*, E Yoshida* and B Salh*

INTRODUCTION

Ischemic colitis is the most common form of intestinal ischemia. Prior studies have characterized this patient population with regards to presenting symptoms, co-morbidities, location of ischemia, surgical operations, and mortality. Our aim was to identify the racial background of patients with colonic ischemia at a Canadian tertiary care centre and determine if there were any disparities in its incidence in various populations.

METHODS

All confirmed biopsy cases of ischemic colitis from 2006 were examined. Patients with IBD, infective colitis, rectal prolapse, NSAID use, and diverticulosis were excluded. 20 cases were available for review and electronic records were searched to characterize this population.

RESULTS

Of the 20 cases of ischemic colitis six were Chinese, one was Vietnamese, and one was Korean. Of the remaining cases 11 were Caucasian and one was Fijian. Thus Asian patients comprised 40% (8/20) of this ischemic population. The 2006 Stats Canada demographics for the referring catchment area showed that the Asian population was 21.1%. Comparing these two groups with Fischer's exact test demonstrated a P value of 0.0597.

The average age of the study group was 73.5 years (range 42 to 89). Hypertension (50%, 10/20) and coronary artery disease (45%, 9/20) were the most common co-morbidities. As in prior studies the most common presenting features were frank rectal bleeding (70%, 14/20) and abdominal pain (65%, 13/20). The majority of patients (80%, 16/20) had non-isolated right colon ischemia. The average length of stay was 10.85 days (range 1 to 84) and the overall mortality was 10% (2/20).

CONCLUSIONS

There was an almost significant higher incidence of ischemic colitis in Asian populations, despite our major limiting factor of the small sample size. Interestingly there were no South Asians with ischemic colitis identified during that year, even though this population has a very high incidence of cardiovascular disease. No definitive conclusions regarding the incidence of ischemic colitis in Asian populations could be made, but this pilot study needs to be repeated with an increased sample size incorporating other centres.

Supervisor: Dr. Bill Salh

Combating Platelet Refractoriness

Dr. Jeffery M Patterson, Dr. Lauren Bolster, Dr. Susan Nahirniak

INTRODUCTION

Defined as an insufficient platelet rise post transfusion, refractoriness remains a significant issue in transfusion medicine. Outside of the risk of life threatening bleeding, it is also a barrier to safely perform therapeutic and diagnostic procedures. One of the most significant mechanisms is immune platelet destruction from HLA anti-bodies. The scope of this quality assurance study is to examine the adequacy of the current protocol utilized by the University of Alberta blood bank in combating this issue.

METHODS

Platelet HLA antibodies were identified in patients using AHG FCT-60 testing, and subsequent platelet transfusions were chosen to avoid these specific antigens. Adequate platelet increments were assessed using a corrected count increment (CCI) calculation, with either a 1-3 hour or 18-24 hour post transfusion platelet count. The mean HLA matched CCI was compared with a mean pooled platelet CCI in each patient to confirm refractoriness. In situations where CCIs could not be calculated, raw platelet count increments were analyzed. Subgroup analysis was also done to see if ABO compatibility of units made a difference in increments.

RESULTS

When assessing 1-3 hour post transfusion platelet counts, 50-56% of transfusion episodes were considered adequate. Transfusion episodes for which a 18-24 hour platelet count was available, 23-25% were sufficient. A negative or no change in platelet values at 18-24 hours was seen in 58% of instances. When ABO matched versus mismatched transfusions were compared, a significant difference in increments was only seen at the 18-24 hour mark.

CONCLUSIONS

Most important in the bleeding patient, or when an invasive procedure is needed, using our current strategy resulted in adequate platelet recovery. Platelet survival was less acceptable. Improved results were seen when ABO matched units were used. Once established, follow-up is key to determine if platelet unit, non-immune or other immune factors are contributing, and should also be addressed.

Supervisor: Dr. Susan Nahirniak

Predictors of chronic lung allograft dysfunction (CLAD) following respiratory virus infection (RVI) in lung transplant recipients

Kieran Halloran, Kathy Jackson, Ali Kapasi, Justin Weinkauff, Dale Lien, Roland Nador

INTRODUCTION

CLAD is a disorder of irreversible, progressive loss in lung function in lung transplant recipients. The two major subtypes, bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS) are leading causes of morbidity and mortality. RVI has been shown to increase risk for BOS and death in lung transplant recipients, though the relationship to RAS is unknown. We examined the characteristics of host response to RVI and whether these predict loss of graft function 1 year post-infection.

METHODS

We identified 49 RVIs in lung transplant recipients occurring 2006 - 2011. The median time from transplantation to RVI was 15 months. Clinical characteristics of host response to RVI selected for this study are listed in Table 1. Spirometric data was collected at pre-infection, time of RVI, and 1 year. The relationship between the characteristics of host response to RVI and loss of graft function, as measured by 10% or greater decrease in FEV1 or FVC, was measured using Fisher Exact testing.

RESULTS

The prevalence of each clinical characteristic of host response to RVI is listed in Table 1. Decline in lung function occurred in 10/49 (20.4%) patients at 1 year, with a mean FEV1 and FVC loss of 21.6% and 13.3% respectively. Isolated FEV1 loss occurred in 6, while FVC loss occurred in 4. Among these, CLAD was documented in 5/10 patients prior to RVI. Hypoxia, radiographic change, hospitalization, subsequent bacterial and fungal colonization were more frequent in these 10 recipients. Only subsequent fungal colonization appears to correlate with lung function decline significantly ($p = 0.02$).

CONCLUSIONS

RVI may contribute to the development or worsening of CLAD. Fungal colonization following RVI is a significant risk factor for loss of graft function at one year. The mechanism for this is unclear and requires further study. Additional univariate and multivariate analysis is planned for this study.

Supervisor: Dr. Roland Nador

Table 1. Features of respiratory virus infection in lung transplant recipients and the relationship to CLAD at 1 year.

Clinical Characteristics	Prevalence n = 49 [%]	Association with CLAD at 1 year (p-value)
Respiratory symptoms	41 [92]	1.00
Acute change in lung function change at time of RV infection (Δ FEV1 or FVC \geq -10%)	23 [47]	0.48
Hypoxia	4 [8]	0.18
Hospitalization	13 [27]	0.10
Radiographic change	6 [12]	0.09
Coexistent bacterial infection	15 [31]	1.00
Subsequent bacterial colonization	5 [10]	0.27
Coexistent Fungal infection	14 [29]	1.00
Subsequent Fungal Colonization	7 [14]	0.02

The Utility of Transient Elastography In Monitoring Patients With Wilson's Disease

Angeli Chopra, Dr. Mang Ma

INTRODUCTION

Transient elastography (Fibroscan; EchoSens, Paris, France) is a non invasive technique involving the acquisition of pulse-echo ultrasound signals to measure liver stiffness in adults and children. Transient elastography values increase proportional to the progression in the histological stage of fibrosis. Patients with Wilson's disease can be difficult to monitor due to the variability of urine copper excretion and the liver biochemical profile does not reflect disease progression accurately. We present a spectrum of distinct cases of Wilson's disease illustrating the usefulness of transient elastography as a potential useful tool to monitor Wilson's disease.

METHODS

4 patients with liver biopsy and ATP7B gene proven Wilson's disease were monitored with standard of care and transient elastography yearly. These patients have been followed from 2008 to 2011. Their clinical course, response to therapy (zinc, penicillamine, or trientine) and compliance were assessed with clinical presentation, detail history, liver tests and repeat liver transient elastography measurements. Transient elastography was performed as per standard protocol.

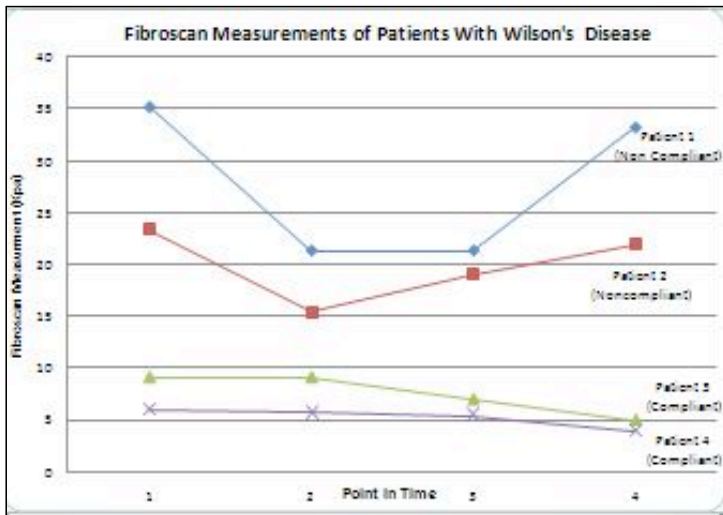
RESULTS

Four Wilson's disease cases (3 males and 1 female) with a mean age of 29.75 +/-6yrs (range 21-37yrs) were reviewed. These patients had hepatic and neurologic/psychological manifestations. Neurological manifestations seen were difficulty with speech articulation, abnormal posturing and fine motor involvement. Psychiatric manifestations included symptoms of aggression and depressed mood. One patient was on zinc therapy on presentation and had a history of treatment noncompliance (Patient 1). Another patient had a history of noncompliance due to social situation (Patient 2). These 2 patients had early cirrhosis and transient elastography changes correlated with history of compliance. The other two patients (patients 3 and 4) were compliant and transient elastography results remained stable.

CONCLUSIONS

Wilson's disease can present with many diverse characteristics and monitoring can be difficult. Transient elastography appears to be an effective method to monitor liver disease progression in Wilson's disease patients.

Supervisor: Dr. Mang Ma



AZATHIOPRINE COMPLIANCE AND ADVERSE EVENTS IN A COHORT OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Isaac Soo MD, Sunny Randhawa BSc, Richard N. Fedorak MD

INTRODUCTION

Leukopenia and elevated liver enzymes (ELE) are adverse effects of azathioprine therapy. Our goal is to characterize compliance with monitoring bloodwork and incidence of adverse effects in a cohort of patients with inflammatory bowel disease (IBD) on azathioprine.

METHODS

Retrospective chart review of inflammatory bowel disease patients initiated on azathioprine from 2007 to 2011

RESULTS

65 patients were diagnosed with Crohn's disease, 38 with ulcerative colitis and two with indeterminate colitis. Average age was 36.7 ± 14.2 . 57 Patients were male. Patients were followed for an average of 804 ± 420 days. Average completion rate of weekly bloodwork (#/4) following the initiation of azathioprine was 2.4 ± 1.7 . Average completion of monthly bloodwork was $57.5 \pm 28.4\%$. There was no significant difference in compliance between therapy started in-hospital versus as an outpatient. 10 (9.5%) patients experienced $WBC \leq 3.0$. Six patients had one while four patients had two(2), three(1) and four episodes(1) of leukopenia. All patients had leukopenia within one year of either: azathioprine initiation, addition of 5-ASA therapy or escalation of azathioprine dosage. Four patients had leukopenic events beyond one year of azathioprine initiation. Two patients had viral illnesses, and the remainder experienced leukopenic events with one year of therapy initiation. Six patients were found to have ELE. All episodes occurred within one year of azathioprine initiation or dose escalation. No significant illness occurred from the adverse events. The weekly and monthly compliance rates for bloodwork were not significantly different from the rest of the cohort in those patients with adverse effects.

CONCLUSIONS

Compliance with serial bloodwork in monitoring leukopenia and ELE in patients with IBD is generally poor. The majority of patients who have detection of leukopenia or ELE are detected within one year of therapy. Patients who do not manifest leukopenia or ELE within this period may not benefit from a prolonged duration of surveillance bloodwork

Supervisor: Dr. Richard N Fedorak

Splenic artery embolization: A novel application in Palliative Care. Two case studies.

Dr. Amanda J Brisebois, Dr Ronald J Brisebois

INTRODUCTION

Pancreatic adenocarcinoma can increase risk of splenic vein thrombosis, which has multiple complications. Splenic artery embolization can improve palliative management in these patients, despite the appearance of being overly aggressive.

METHODS

Two cases that were encountered were reviewed. Analysis of patient presentation and management follows.

RESULTS

A 42 year old presented with blood loss from gastric varices. Investigation revealed an unresectable Stage IV pancreatic cancer. Splenic vein thrombosis was the determined etiology of the gastric varices. He received 12 units of blood, repeat endoscopies and sclerotherapies with minimal benefit. Splenic embolization was performed, and bleeding stopped within 24 hours. The second patient was found to have a pancreatic mass, and liver lesions. FNA confirmed stage IV inoperable pancreatic adenocarcinoma. Palliative chemotherapy was offered, but his platelets dropped to a level that precluded chemotherapy. The thrombocytopenia was determined to be secondary to splenomegaly caused by splenic vein thrombosis. Referral to a surgeon was made in hopes of a splenectomy. Splenic artery embolization was undertaken, and the patient's platelets returned to a level to allow palliative chemotherapy. The embolizations were performed by experienced interventional radiologists, at different centers in Edmonton. Both embolizations produced minimal adverse effects.

CONCLUSIONS

Splenic vein thrombosis secondary to malignancy is not an uncommon phenomenon. Bleeding or thrombocytopenia caused by this can preclude palliative therapies and contribute to earlier death. Splenic artery embolization should be considered as a potential adjunctive palliative therapy. It may minimize the need for transfusion, stop bleeding, or increase platelets to allow further palliative chemotherapy.

Supervisor: Dr. Ronald Brisebois

SAMI: St Albert Myocardial Infarction Project

Ann Knauer, RN BN and Danielle Gri RN BScN

INTRODUCTION

During the period of Sept 2010 to August 2011, the St Albert and Sturgeon Primary Care Network (SASPCN) and the Sturgeon Community Hospital Coronary Care Unit (SCH CCU) collaborated on a pilot project titled St Albert Myocardial Infarction (SAMI). The SAMI project identified Myocardial Infarction (MI) patients early post CCU discharge and aimed to transition them through the six week wait to cardiology follow-up and formal cardiac rehabilitation (CR). This is a time in the patient journey when one is vulnerable and motivation is potentially high.

METHODS

As patients are discharged from SCH CCU a detailed referral is sent to the SASPCN. The goal is to have each patient contacted by a Registered Nurse (RN) within 72 hours to offer initial support. The intent is to reinforce discharge information, educate, assist with lifestyle modification, and ensure medications are being taken appropriately. As well, follow-up with family physician, specialists and SASPCN team are facilitated. All patients are strongly encouraged to attend CR.

RESULTS

Data to be presented includes patient demographics, risk factors, successes and challenges. Most of the SAMI patients attended CR and those who did not were still well connected to the PCN team. Patients admitted to SCH CCU come from all geographic areas and do not all belong to SASPCN. This meant many patients were not eligible to participate in SAMI. There is potential for greater success if all PCNs and hospitals in the Zone considered adopting similar programs.

CONCLUSIONS

MI patients in Edmonton wait roughly 6 weeks after CCU discharge to enter CR. PCNs are well positioned to fill this important gap in care. SAMI is a simple, cost-effective process which has shown that continuity of care between acute and primary care significantly improves patient outcomes.

Supervisor: Dr. Zaheer Lakhani

Antiviral Responses of Human Mast Cells to Influenza A Infection

Tae Chul Moon, Candy W. Marcet, Chris D. St Laurent, Jordan M. Wiebe, Nav Singh, A. Dean Befus

INTRODUCTION

Influenza virus (Flu) causes a febrile respiratory disease, and seasonal Flu results in significant morbidity and mortality with about 500,000 deaths worldwide every year. Flu infects predominantly airway epithelial cells (EC) and does not normally spread to other tissues. Protective host defenses against Flu are incompletely understood.

It is now recognized that mast cells (MC) are important in innate and acquired immunity against bacterial, fungal, and viral infections, in addition to classical roles in allergic inflammation and anti-helminth immunity. MC are widely distributed and abundant at mucosal surfaces, in prime locations to encounter microbes and alert the immune system.

We hypothesized that MC can be infected by FluA and contribute to host defense against FluA infection.

METHODS

Human MC lines (HMC1 and LAD2) and primary MC cultured from human peripheral blood progenitors (PBMC) were exposed to FluA (PR/34/8, H1N1, 20-100HAU/ml). Human epithelial cell line (Calu-3) was used as comparative cell type. The viral RNA, complementary RNA, viral transcripts, viral sensors, and antiviral proteins in the infected cells were determined by RT-PCR and Western blot. New virus release and its infectivity were measured using hemagglutination and hemadsorption assay, respectively.

RESULTS

We detected viral sensors (RIG-I, MDA-5), FluA gene transcription, replication and protein synthesis, and antiviral proteins (PKR, MxA, ISG-15, p56, viperin) in human MC. However, there were distinct kinetics in viral RNA and antiviral protein expression between MC and EC. There was no significant release of infectious FluA from MC, whereas EC produce ~100-fold more viral particles compared with the inoculating dose. Moreover, MC-EC coculture protects EC from FluA infection by limiting the release of FluA particles, as well as reducing EC death.

CONCLUSIONS

MC have antiviral mechanisms and communicate with EC to restrict FluA replication, both of which are likely to contribute to host immunity.

Funding: The Lung Association; Canadian Institutes of Health Research; Walter & Jessie Boyd and Charles Scriver MD/PhD Studentship to CM

Supervisor: Dr. Dean Befus

Beta-fructans reduce inflammation in mild to moderate ulcerative colitis through specific microbiota changes associated with improved butyrate formation and Muc2 expression

Rosica Valcheva¹, Petya Koleva^{1,2}, Bartholomeus J. Meijer¹, Jens Walter³, Michael Gänzle², Levinus A. Dieleman¹

INTRODUCTION

Dysbiosis of intestinal microbiota is important for the pathogenesis of ulcerative colitis (UC) in genetically susceptible individuals. Non-digestible, fermentable carbohydrates, particularly fructans, stimulate growth and activity of specific protective microbiota. Therefore fructans may reduce disease activity by promoting “healthier” gut microbiota. The aim of the present study is to assess if fructans can reduce inflammation in UC patients and to identify their protective mechanisms.

METHODS

Patients with mild to moderate active UC, on stable doses of oral 5-ASA, were randomized to 7.5g or 15g daily oral inulin plus fructo-oligosaccharides (FOS) (1:1) for 9 weeks. The clinical response was assessed by UCDAI, endoscopic activity and fecal calprotectin at week 0 and week 9. Multiplex pyrosequencing of 16S rRNA tags was employed to characterize mucosal and fecal microbiota. Colonic butyrate metabolism was studied by measuring fecal butyrate, the bacterial butyryl-CoA-transferase genes abundance, and the butyrate monocarboxylate transporter 1 (MCT1) expression. Mucin 2 (MUC2) relative expression was quantified as well. Statistical analysis using the Mann-Whitney and Spearman tests and principle component analysis were performed to reveal the effect of fructans on the intestinal microbiota, Muc2 and disease activity.

RESULTS

Twenty four patients completed the trial; 12 patients (50%) showed a clinical response (UCDAI decrease of 3 or more), 10 of them (42%) entered clinical remission (UCDAI less than 2). Fructans showed a dose-dependent effect; patients on 15 g dose had an average decrease in UCDAI of 2.5, versus a decrease of 1.2 in the 7.5 g dose. The clinical response was associated with an increase of bacterial biodiversity. Inflammation was associated with *Leuconostoc*, *Enterococcus*, *Flavobacterium* and genera from β - and γ -Proteobacteria. In contrast colitis reduction was correlated to increased *Faecalibacterium*, *Roseburia*, *Dialister* and *Lactobacillus*. The clinical response was associated with improved butyrate production in the colon and upregulation of MCT1 expression. This suggests that enhanced butyrate formation by fructans mediates colitis reduction. Furthermore, downregulation of MUC2 expression was correlated with disease-associated bacterial taxa. This indicates that Muc2 depletion associated with specific mucosal microbiota could mediate ulcerative colitis.

CONCLUSIONS

Fructans reduce ulcerative colitis through specific microflora changes associated with improved butyrate formation and reduced Muc2 depletion. This small open label nine week treatment study indicates that fructans show promise as adjunctive therapy to induce remission in UC.

Supervisor: Dr. Levinus A. Dieleman

Increased intestinal permeability among first-degree relatives of Crohn's patients is not associated with increased mucosal ulcerations on small bowel video capsule endoscopy.

Christopher Teshima, Mohamed El-Kalla, Samina Turk, Ronda Blasco, Marilyn Gordon, Peter Ho, Amanda Mullins, Daniel Wong, Jonathan Meddings, Hien Huynh, Levinus A. Dieleman

INTRODUCTION

First-degree relatives (FDR) of Crohn's disease (CD) patients have highest risk for developing CD. CD patients and a substantial portion of FDR have increased intestinal permeability. It is unclear whether FDR have abnormal permeability because of early, asymptomatic CD or whether this occurs without mucosal inflammation. Video capsule endoscopy (VCE) is the most sensitive means of imaging the small intestine and can identify mucosal ulcerations suggestive of subclinical CD. The purpose of our study was to determine if abnormal small intestinal permeability in healthy FDR is associated with small bowel mucosal abnormalities detected by VCE.

METHODS

349 CD patients consented to have their FDR between 10-45 years of age contacted regarding study participation. Eligible FDR underwent small bowel permeability testing as measured using the lactulose/mannitol (L/M) test that is based on the fractional urinary excretion of these sugars. FDR with abnormal permeability were compared to FDR with normal small permeability by VCE to assess for small bowel inflammatory changes. The primary outcome was the number of mucosal ulcerations seen on VCE in each permeability group.

RESULTS

175 FDR consented to participate and completed the permeability test. 37 (21%) had abnormally increased permeability. 38 subjects with normal and 23 subjects with abnormal permeability underwent VCE. On VCE, there was no difference in small bowel mucosal abnormalities with a mean of 2.29 (range 0-16) ulcers in the normal and 1.56 (range 0-10) ulcers in the abnormal permeability groups respectively (N.S).

CONCLUSIONS

There is no apparent association between small bowel ulcerations seen on VCE between healthy, asymptomatic FDR with abnormally increased intestinal permeability and FDR with normal permeability. Thus, the increased small bowel permeability in FDR does not seem to be caused by subclinical CD and may possibly relate to an earlier stage affecting the tight junctions comprising the gut barrier.

Supervisor: Dr. Leo Dieleman

One Third Of Patients Treated With Adalimumab Or Infliximab For Crohn's Disease Or Ulcerative Colitis Permanently Dose-Escalate Due To Loss Of Response

Darryl Fedorak, Pam Osatiuk, Ibrahim Quazi, Karen Wong, Karen Kroeker, Leo Dieleman, Richard N. Fedorak

INTRODUCTION

Inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, are chronic relapsing inflammatory conditions of the intestine, often in young adults, characterized by abdominal pain and diarrhea. The introduction of tumor necrosis factor (TNF)-blockade with infliximab (IFX) and adalimumab (ADA) has offered significant advances in the treatment of refractory IBD and its complications.

METHODS

Through a hand searched chart review, patients were eligible to be included in the cohort for this study if they had: (1) responded to IFX (5mg/kg) induction dosing at wks 0, 2, and 6 or ADA induction dosing of 160mg, 80 mg at wks 0 and 2, respectively and, (2) advanced onto scheduled maintenance treatment every 8 weeks with IFX or every 2 wks with ADA and, (3) achieved a stable corticosteroid-free clinical benefit for at least 6 months and, (4) lost response to the anti-TNF therapy and, (5) had sufficient follow-up that allowed assessment of ongoing wellness and/or disease relapse with escalated therapy.

RESULTS

363 patients met criteria and were included in this study. At the time of analysis 187/287 (65%) of IFX-treated patients were still in remission on 5mg/kg every 8 wks; while 55/76 (72%) of ADA-treated patients were still in remission on 40mg every other wk. Kaplan Meier survival analysis demonstrated that 100/287 (35%) of IFX-treated patients required dose escalation to 5mg/kg every 4 wks (median time to dose escalation 46.6 mo (CI 37.1, 1.6)), while 21/76 (28%) of ADA-treated patients required dose escalation to 40mg weekly (median time to dose escalation 67 mo (CI 28.5, NA)). De-escalation of dose was uncommon; 7 IFX patients and zero ADA patients de-escalated to the original doses and maintained remission. The results for loss of response and dose escalation were similar for CD and UC.

CONCLUSIONS

Approximately one third of patients with IBD treated with an anti-TNF agent lost response and required dose escalation in order to regain response. The median time to reach dose escalation was longer for adalimumab than for infliximab. Following dose escalation only very few patients were de-escalated to the original dose.

Supervisor: Dr. Richard N. Fedorak

