National CIHR Research Training Program in Hepatitis C
Subvention nationale de formation des IRSC sur l'hépatite C

Canadian Symposium on Hepatitis C Virus

Symposium canadien sur le virus de l'hépatite C

February 23, 2012 - 23 février 2012

Hilton Bonaventure Hôtel, Montréal, Québec

CHUM
CENTRE DE RECHERCHE

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Welcome Message

Dear colleagues,

We would like to welcome each and every one of you to the Canadian Symposium on Hepatitis C Virus (HCV). Over the past 10 years, Canadian researchers have contributed to major discoveries in the field of Hepatitis C. Such discoveries include: the first proof of concept antiviral against HCV, the development of the first humanized mouse model for HCV infection, identification of novel biomarkers for HCV disease progression and treatment outcome and research assessing access to treatment in marginalized populations, in particular individuals infected with HIV, injection drug users (IDUs), and first nations people. Despite this internationally recognized success, it is evident that interactions between Canadian scientists and clinicians need to be strengthened in order to effectively respond to current and future challenges in the management of the disease. We believe that a Canadian conference on HCV is timely and will provide an ideal forum to exchange research findings, promote collaborators, and create synergy among Canadian investigators. This is the first of what we hope will become an annual forum for exchange among all Canadian investigators working on HCV and knowledge translation for healthcare practitioners and community based groups.

The National Canadian Research Training Program in Hepatitis C (NCRTP-HepC) has significantly contributed to advancing research training and knowledge translation in the field of hepatitis C. It has been very successful in its goal to improve research capacity by developing a network of collaborative investigators whose work encompasses the social, behavioural, clinical and basic sciences, crucial to develop, study and implement effective prevention and care programs to eradicate HCV-related diseases in Canada and worldwide. It was only befitting that the NCRTP-Hep C would take the lead on organizing this conference.

We would like to welcome you to this meeting and to our beautiful city of Montreal. We look forward to finding out about your exciting research and to discussing together how we can shape the future of Hepatitis C research in Canada.

The organizing committee,

Message d'accueil

Chers collègues,

Nous vous souhaitons la bienvenue au Symposium canadien sur l'hépatite C. Au cours des 10 dernières années, des chercheurs de partout au Canada ont contribué à certaines des découvertes majeures dans le domaine de l'hépatite C. Ces découvertes incluent la première démonstration clinique de l'efficacité de molécules antivirales ciblées contre le virus de l'hépatite C (VHC), le développement d'un modèle de souris humanisée de l'infection par le VHC, l'identification de nouveaux biomarqueurs de la progression de la maladie et les déterminants de l'accès au traitement antiviral chez les populations marginalisées, notamment les individus infectés par le VIH, les utilisateurs de drogues illicites (UDI) et les citoyens faisant partie des Premières nations. Malgré ces succès reconnus internationalement, il est apparu évident que les interactions entre les scientifiques et les cliniciens canadiens avaient besoin d'être renforcées afin de répondre efficacement aux défis présents et futurs que soulèvent l'infection par le VHC. Nous croyons que la mise sur pied d'une conférence canadienne sur le VHC était devenue nécessaire de façon à disséminer les résultats de la recherche, promouvoir les collaborations, offrir un forum d'échange et créer une synergie entre les chercheurs canadiens de tous les horizons. Il s'agit du premier pas de ce qui, nous l'espérons, deviendra une rencontre annuelle pour tous les chercheurs canadiens qui travaillent sur le VHC et pour tous les professionnels de la santé et les groupes communautaires intéressés à élargir leurs connaissances dans ce domaine.

Le Programme de subvention nationale de formation des Instituts de recherche en santé du Canada sur l'hépatite C (NCRTP-HepC) a contribué de façon significative à la formation de nouveaux étudiants et à la diffusion des connaissances dans le domaine de l'hépatite C. En particulier, ce programme a permis d'augmenter le nombre d'étudiants et l'étendue des travaux de recherche en développant un réseau de chercheurs dont les thèmes englobent les sciences sociales et du comportement ainsi que la recherche clinique et fondamentale. Les objectifs du programme sont, entre autres, de développer, étudier et mettre en œuvre des moyens efficaces de prévention et des programmes de soins dans le but d'éradiquer à long terme l'infection par le VHC au Canada et dans le reste du monde. Il était donc de mise que le NCRTP-Hep C prenne l'initiative de l'organisation de cette conférence.

Nous tenons à vous souhaiter la bienvenue à Montréal. Espérons que nous pourrons découvrir les problématiques importantes que soulèvent en ce moment l'infection par le VHC, l'étendue des travaux de recherche qui sont actuellement effectués sur le VHC au Canada et en profiter afin de discuter ensemble des moyens à prendre afin de façonner l'avenir de la recherche sur l'hépatite C au Canada.

Le comité organisateur,

Université de Montréal

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Direction du développement professionnel continu

Accréditation

La Direction du Développement professionnel continu (DPC) de la faculté de médecine de l'Université de Montréal est pleinement agréée par le Comité d'agrément de l'éducation médicale continue (CAÉMC) et par le Collège des médecins du Québec (CMQ).

La Direction du DPC reconnaît, à la présente activité, 7 heures créditées de catégorie 1 (Main Pro-M1) pour les médecins omnipraticiens (médecin de famille) présents.

Pour les médecins spécialistes membres du Collège Royal des médecins et chirurgiens du Canada (CRMCC), la Direction du DPC reconnaît 1 crédit de la section 1 par heure de participation pour un total de 7 crédits pour l'activité globale conformément au programme du maintien du certificat du CRMCC.

Pour tout autre professionnel participant, ce programme donne une attestation de participation de 7 heures.

Les participants doivent réclamer un nombre d'heures conforme à leur participation.

Accreditation

The Division of Continuing Professional Development (CPD) of the Faculty of Medicine of the Université de Montréal is fully accredited by the Committee on Accreditation of Continuing Medical Education (CACME), by the Collège des médecins du Québec (CMQ).

The Division du CPD approves this activity for 7 hours of category 1 (Main Pro-M1) credits for the attending general practitioner (family physician).

For the specialist physician, the Division of CPD approves 1 credit per hour of attendance for a total of 7 credits for the entire activity in accordance with the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada (RCPSC).

For all other participants, this program grants a certificate of attendance of 7 hours.

Participants should claim a number of hours consistent with their attendance.

Program – Programme

07h00 - 08h00 Breakfast, Registration, Exhibition and Poster Area Opens

08h00 - 08h15 Welcome and Introductions
Dr. Marc Bilodeau, Université de Montréal, Montréal, Canada

Biomedical Sciences
Chairs: Dr. Michael Houghton, Dr. Christopher Richardson

08h15 - 08h45 The Virology of Hepatitis C Virus
Prof. Ralf Bartenschlager, University of Heidelberg, Heidelberg, Germany

08h45 - 09h00 The Elusive Role of HCV p7 in Virus Production
Dr. Rodney Russell, Memorial University, St. John's, Canada

09h00 - 09h15 Y-Box-Binding Protein-1-Containing Ribonucleoparticle Hijacking During Hepatitis C Virus Life Cycle
Laurent Chatel-Chaix, IRIC, Université de Montréal, Montréal, Canada

09h15 - 09h30 The Roles of Fitness and the Genetic Barrier in the Context of Synonymous Nucleotide Changes during HCV Replication
Robert A. Kozak, McGill University, Montréal, Canada

09h30 - 09h45 T Cell Immunity in HCV/HIV Co-Infection
Dr. Mario Ostrowski, University of Toronto, Toronto, Canada

09h45 - 10h00 Modulation of HCV Specific CD8 T Cells Exhaustion and Survival by IL-21 During Acute Hepatitis C.
Hassen Kared, CRCHUM, Université de Montréal, Montréal, Canada

10h00 - 10h15 Does a Vaccine Derived From a Single HCV Strain Elicit Broad Cross-Neutralizing Antibodies in Humans?
John Law, University of Alberta, Edmonton, Canada

10h15 - 10h45 Coffee Break

Behavioural Sciences
Chairs: Dr. Gerry Mugford, Dr. Benedikt Fischer

10h45 - 11h15 New Socially-Based Approaches To HCV Prevention
Prof. Samuel Friedman, National Development and Research Institute, New York, USA

11h15 - 11h30 Psychological Issues in the Care of People Living with Hepatitis C
Dr. Louise Balfour, The Ottawa Hospital at The University of Ottawa, Ottawa, Canada

11h30 - 11h45 General Consideration Towards Reducing the Burden of HCV in Drug User Populations
Dr. Benedikt Fischer, Simon Fraser University, Vancouver, Canada

11h45 - 12h00 Nothing for us, Without us: the Meaningful Involvement of Clients in HCV Treatment and Prevention.
Kate Mason, Research Coordinator South Riverdale Community Health Centre

12h00 - 13h00 Lunch, room Fontaine CDE and Côte St-Luc

Epidemiology and Public Health
Chairs: Dr. Jason Grebely, Dr. Marina Klein

13h00 - 13h30 HCV Prevention and Treatment: Progress & Challenges
Dr. Shruti Mehta, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

13h30 - 13h45 Population-Based Health Outcomes in Patients with HCV: Implications for a Public Health Response
Dr. Mel Krajden, BCCDC, Vancouver, Canada

13h45 - 14h00 Illicit Prescription Opioids and HCV Transmission Among Injection Drug Users
Dr. Julie Bruneau, Université de Montréal, Montréal, Canada

14h00 - 14h15 Female Sex and Variations in IL28B are Independently Associated with Spontaneous Clearance of Acute HCV Infection.
Jason Grebely, The Kirby Institute for Infection and Immunity in Society, University of New South Wales, Sydney, Australia

14h15 - 14h30 Long-Term Direct Health Care Costs Attributable to Hepatocellular Carcinoma; A Population-Based Study
HH Thein, Dalla Lana School of Public Health, University of Toronto, Toronto, ON

14h30 - 15h00 Coffee Break

Clinical Sciences
Chairs: Dr. Lorne Tyrrell, Dr. Raymond Tellier

15h00 - 15h30 Directly Acting Antivirals for HCV and Resistance
Prof. Jean-Michel Pawlotsky, Université Paris Est, Créteil, France

15h30 - 15h45 Non Invasive Methods for the Assessment of HCV-Related Liver Fibrosis: Challenges and Controversies
Dr. Rob Myers, University of Calgary, Calgary, Canada

15h45 - 16h00 Canadian Guidelines for the Treatment of HCV in Injection Drug Users
Dr. Brian Conway, University of British Columbia, Vancouver, Canada

16h00 - 16h15 Donor Interleukin-28B Polymorphisms are Associated with Hepatic Fibrosis and Inflammatory Molecular Changes Following Liver Transplantation in Patients with Hepatitis C Infection
Brad Thomas, University of Alberta, Edmonton, Canada

16h15 - 16h30 Pegylated Interferon-Associated Retinopathy is Frequent in HCV Patients with Hypertension and Justifies Ophthalmologic Screening
Giada Sebastiani, McGill University, Montréal, Canada

16h30 - 17h00 Canadian Symposium on Hepatitis C Virus Special Achievement Award
Dr. Jenny Heathcote, University of Toronto, Toronto, Canada

17h00 - 17h15 Closing Remarks
Dr. Naglaa Shoukry, Université de Montréal

17h15 - 19h15 Cocktail and Poster Viewing
Room Fontaine CDE

Committees – Comités

Organizing Committee - Comité organisateur

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Julie Bruneau, Université de Montréal
Matthias Götte, McGill University
Jason Grebely, University of New South Wales
Michael Houghton, University of Alberta
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Speakers Biographies and Abstracts – Biographies des conférenciers et résumés

Biomedical Sciences

Prof. Ralf Bartenschlager, University of Heidelberg, Heidelberg, Germany

Biography

Ralf Bartenschlager's research interest is the study of the replication of the two flaviviruses Dengue virus (DENV) and hepatitis C virus (HCV). Following the establishment of HCV cell culture systems he now investigates virus-host interactions and the underlying mechanisms of the interference of viral proteins with host cell functions including the block of innate antiviral defense and its possible link to persistence. Another aspect is the biogenesis and 3D architecture of the cytoplasmic virus-induced replication factories.
Ralf Bartenschlager studied Biology at the University of Heidelberg where he received his PhD degree in Molecular Biology in 1990. After a short post-doctoral training at the Center for Molecular Virology in Heidelberg he joined the Central Research Unit at the Hoffmann-La Roche AG in Basel as a postdoctoral researcher from 1992 till 1993. Re-entering academia he worked as a research assistant at the University of Mainz, where he habilitated in 1999 and became full professor for Molecular Virology in 2001. Since 2002 he is full professor and head of the Molecular Virology unit in the Department of Infectious Diseases at the University of Heidelberg.
Over the past years he is a steering committee member of several EU- and DFG-funded networks and speaker of the Forschergruppe FOR1202.

Abstract

The Virology of the Hepatitis C Virus

Persistent infection with the hepatitis C virus (HCV) is a major global health problem. Around 2-3% of the world population are chronically infected and these individuals are at high risk of developing steatosis, fibrosis and liver cirrhosis. The latter is a major predisposing factor for development of hepatocellular carcinoma (HCC).
The HCV genome contains a single large open reading frame encoding for a polyprotein that is cleaved into 10 different products. To all of these proteins distinct functions could be ascribed and the 3D X-ray crystal structures of several of these proteins have been resolved. With the advent of adequate cell culture models, new insights into the intimate interaction between HCV and the host cell have been gained. For instance, several molecules involved in viral entry have been identified and a complex picture emerges how the virus productively infects a cell. Likewise, new and surprising insights have been gained how HCV particles assemble. It turned out that lipid droplets play a very important role for virion morphogenesis, and that NS5A appears to be a key regulator of the different steps of the viral replication cycle. Finally, high-throughput screening methods have been used to identify host cell factors that contribute to HCV replication. Two prominent examples are cyclophilinA that seems to activate the viral replicase and phosphatidyl-inositol kinases that appear to contribute to the formation of the viral membranous replication complex. The common denominator is that HCV usurps multiple cellular processes to achieve efficient RNA replication, virus assembly and virion infectivity.

Dr. Rodney Russell, Memorial University, St. John's, Canada

Biography

Rod Russell completed his undergraduate training in Biochemistry at Memorial University of Newfoundland in 1996, followed by a Master's degree in HIV Immunology under the supervision on Dr. Michael Grant. In 1999 he moved to McGill University to work with Drs. Chen Liang and Mark Wainberg on virus assembly and genome packaging in HIV. From there he went to the National Institutes of Health to carry out Postdoctoral Research on the hepatitis C virus (HCV) under the supervision of Drs. Sue Emerson and Bob Purcell. During his time at the NIH Dr. Russell was involved in the establishment of the first fully infectious HCV cell culture system. In January of 2008 he was recruited as an Assistant Professor at Memorial University and established an independent research program aimed at studying the Molecular Virology and Viral Immunology of HCV. His work mainly focuses on identifying viral and cellular proteins involved in the production of infectious hepatitis C viral particles. Other projects ongoing in his laboratory involve the adaptation of the HCV JFH1 cell culture system to a microtiter plate format that allows the study of the effects of HCV-infected cells on the function of various subsets of immune cells. Along with colleagues at McGill University Dr. Russell has initiated a project aimed at studying the development of drug resistance in HCV. His work is funded by Operating Grants from the Canadian Institutes of Health Research (CIHR), and Dr. Russell is the recipient of a CIHR New Investigator Award

Abstract

The Elusive Role of HCV p7 in Virus Production

The HCV genome encodes a 63aa protein, p7, which is located between the structural and non-structural proteins. P7 localizes to endoplasmic reticulum membranes and is composed of two transmembrane domains and a cytoplasmic loop. The role of p7 is relatively unknown and is currently classified as neither a structural nor non-structural protein. Several studies have shown that p7 acts as an ion channel and is crucial for infectious virus production in cell culture as well as infectivity in chimpanzees. Recently, it was suggested that p7 may prevent acidification of intracellular compartments that may otherwise render assembled HCV particles non-infectious. The aim of this study was to investigate whether p7 has additional roles in virus assembly and/or release. Accordingly, we created multiple alanine triplet mutants along one third of the p7 coding region. We observed that mutations in these regions decreased infectious virus production with variable efficiencies and these results were confirmed using a single-cycle virus production assay. Analysis of intra- and extracellular virus titres indicated that p7 functions at a stage prior to generation of infectious particles. These effects were not due to altered RNA replication, since no effect on NS3 or NS5A protein expression was observed. Confocal microscopy analyses revealed that the p7 mutations did not affect recruitment of core protein to lipid droplets. Additionally, these mutants could still form dense higher order structures, as determined by density gradient fractionation, suggesting that p7 acts after putative nucleocapsid formation. The results of this study will provide a better understanding of the function of p7 in virion morphogenesis.

Dr. Mario Ostrowski, University of Toronto, Toronto, Canada

Biography

Mario Ostrowski is associate professor of Medicine, Immunology and pathobiology and lab medicine at University of Toronto and also is a consultant infectious diseases clinician at St. Michael's Hospital in Toronto, Canada. His interests include studies on T cell immunoregulation in HIV and HCV infection, molecular adjuvants for vaccination, pDC-virus interactions, and the role of endogenous retroviruses in HIV infection.

Abstract

T Cell Immunity in HCV/HIV Co-Infection

Individuals co-infected with HIV and HCV have a more rapid progression of liver disease and cirrhosis. Potent CD4 and CD8 T cell immune responses are associated with control of both viruses. Inflammation induced by intra-hepatic T cells has been proposed to induce fibrosis in HCV infection. It has been puzzling why HCV disease progresses faster in the setting of HIV co-infection if HIV induces a state of CD4 suppression. A number of studies have suggested that HIV induces a state of T cell dysregulation resulting high level HCV replication and a pro-inflammatory milieu in the liver leading to fibrogenesis. In HIV co-infection, the following mechanisms likely contribute to enhanced pathogenesis: 1) HIV infection reduces CD4 help of HCV specific CD8 T cell responses, 2) HIV co-infection enhances the levels of the CD8 T cell exhaustion markers, PD-1 and Tim-3 on HCV specific CD8 T cells, 3) HIV specific CD8 T cells that produce TNF-a are recruited to the liver increasing inflammation, 4) HIV-specific CD8+ T cells develop in coinfected persons that recognize molecularly similar HCV peptides, albeit at a lower affinity, 5) HIV-related depletion of the gut epithelium may lead to the loss of HCV-specific CD4+ T cells as well as the translocation of microbial products such as LPS, which induces the expression of profibrogenic cytokines. The effect of HAART on reducing some of these effects and restoring HCV specific immunity will be discussed. In addition, new findings on the role of CD4 cytokines and HIV co-infection will be presented.

Behavioural Sciences

Prof. Samuel Friedman, NDRI, New York, USA

Biography

Samuel R. Friedman is Director of HIV/AIDS Research at National Development and Research Institutes, Inc. and the Director of the Interdisciplinary Theoretical Synthesis Core in the Center for Drug Use and HIV Research, New York City. He also is associated with the Department of Epidemiology, Johns Hopkins University, and with the Dalla Lana School of Public Health, University of Toronto. Dr. Friedman is an author of about 400 publications on HIV, hepatitis C, STI, and drug use epidemiology and prevention. Honors include the International Rolleston Award of the International Harm Reduction Association (2009), the first Sociology AIDS Network Award for Career Contributions to the Sociology of HIV/AIDS (2007), and a Lifetime Contribution Award, Association of Black Sociologists (2005). He has published many poems in a variety of publications and a book of poetry (*Seeking to make the world anew: Poems of the Living Dialectic*. 2008. Lanham, Maryland: Hamilton Books).

Abstract

New approaches to HCV prevention

Friedman, Samuel R; Mateu-Gelabert, Pedro; Sandoval, Milagros
National Development and Research Institutes, Inc., New York, NY

HCV prevention has proven to be very difficult—yet a considerable proportion of people who have injected drugs for 8 years or more nonetheless remain uninfected. We developed a new research design, the positive deviance case-control life history approach, to develop hypotheses about how people who inject drugs (IDUs) had remained uninfected. In this design, we compare in-depth interviews about the life histories, social and behavioral lives, and strategies of long-term IDUs who were negative on HCV (and also HIV in some versions) with similar participants who were HCV+. (Collaborators are conducting similar Staying Safe projects in several countries, including Canada.) We developed hypotheses that, in addition to standard behavioural strategies, IDUs would be more likely to remain uninfected to the extent that they successfully pursued "symbiotic goals" such as avoiding withdrawal, maintaining social relations with other people, and maintaining enough income to be able to remain housed and also afford their needed drugs. Additional protective mechanisms included planning skills and helping network members adapt protective skills. Dr. Mateu-Gelabert conducted a pilot intervention to teach IDUs Staying Safe strategies that led to significant declines in self-reported risk behaviours. We also have developed a reliable Staying Safe questionnaire appropriate for cohort studies to study whether engaging in actions designed to achieve symbiotic goals leads to less risk behavior and to lower rates of new HCV and HIV infection.

Funding source (f): US National Institute on Drug Abuse grants R01 DA006723 Social Factors and HIV Risk and R01 DA DA019383-01A1 Staying Safe: Long-term IDUs who have avoided HIV & HCV

Dr. Louise Balfour, The Ottawa Hospital at The University of Ottawa, Ottawa, Canada

Biography

Dr. Louise Balfour is currently an Associate Professor with the Division of Infectious Diseases in the Faculty of Medicine at The University of Ottawa. She has also worked as an HIV clinical health psychologist at The Ottawa Hospital since 1996.

Dr. Balfour obtained her Bachelor's degree in Montreal at McGill University and her Masters and Ph.D. in Clinical Psychology at Concordia University. Dr. Balfour currently holds several CIHR research grants and she has an active Psychological Behavioural Medicine Research Lab at The Ottawa Hospital.

Dr. Balfour's program of clinical research focuses on helping HIV and HCV patients with their readiness for starting treatment, increasing their HV and HCV treatment knowledge, improving their medication adherence, helping patients cope with symptoms of depression and reducing HIV and HCV related stigma. Dr. Balfour is also currently conducting a novel pilot study on helping PHAs quit smoking.

Dr. Balfour has also worked on several international HIV collaborations with the goal of improving HIV treatment knowledge and adherence in Guyana, Colombia, and South Africa.

Abstract

Psychological issues in the care of people who are living with Hepatitis C

This presentation will review important psychological issues related to providing optimal treatment care to meet the needs of people who are living with Hepatitis C. Depression, substance use, stigma, and Hepatitis C related treatment knowledge are key factors that need to be systematically assessed and addressed in order to improve HCV treatment uptake and optimize HCV treatment adherence. The clinical implications for helping HIV/HCV co-infected patients cope with the complex psychological and physical issues associated with the double challenge of living with HIV and HCV will also be highlighted and discussed.

Dr. Benedikt Fischer, Simon Fraser University, Vancouver, Canada

Biography

Benedikt Fischer, PhD, is Professor & CIHR/PHAC Chair in Applied Public Health, and Director, Centre for Applied Research in Mental Health and Addictions (CARMHA), in the Faculty of Health Sciences, Simon Fraser University, Vancouver, and Senior Scientist, Social & Epidemiological Research Department, Centre for Addiction and Mental Health (CAMH), Toronto

Abstract

General Consideration Towards Reducing the Burden of HCV in Drug User Populations

This presentation will provide a review several wider socio-behavioral and systemic factors relevant for consideration and knowledge translation towards designing and delivering more effective Hepatitis C Virus (HCV) prevention and treatment measures for the high-risk group of marginalized street drug users.

Epidemiology and Public Health

Dr. Shruti Mehta, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

Biography

Dr. Shruti Mehta is an Associate Professor of Epidemiology at the Johns Hopkins School of Public Health. She received her PhD and MPH from the Johns Hopkins Bloomberg School of Public Health. Her work focuses on injection drug using populations and is broad in scope ranging from understanding the epidemiology of HIV and HCV co-infection to interventions to improve the uptake of HIV and HCV treatment among IDU populations. She currently leads the AIDS Linked to the IntraVenous Experience (ALIVE) study, which is a community-based cohort of IDUs that has been ongoing since 1988. She also has ongoing research efforts in India which focus on non-invasive markers of liver fibrosis, HCV treatment preparedness and integrated care models to improve uptake of HIV testing and treatment.

Abstract

HCV Prevention and Treatment: Progress & Challenges

The burden of HCV in IDU populations is high because 1) HCV is highly transmissible through needle stick; 2) HCV infections spread quickly through IDU populations because of needle sharing; and 3) HCV is highly prevalent in IDU populations. To reduce the spread of HCV, expanded efforts are needed to prevent new infections (by targeting spread through needle sharing) and to treat those already infected (to reduce the prevalence of chronic HCV infection in IDU populations). While there has been much progress on both prevention and treatment fronts, challenges remain and new strategies are needed. In terms of prevention, expanded harm reduction efforts primarily directed at preventing HIV infection have been associated with declines in HCV incidence among IDU populations in many developed country settings. However, declines in incidence have been less robust than those for HIV incidence and there has been limited impact on HCV prevalence among IDU populations. Harm reduction appears to be working but needs to be expanded in order to truly impact population-level burden of HCV. In terms of treatment, 2011 marked a new era in HCV treatment with the approval of two direct-acting antivirals that improved dramatically improved efficacy of treatment in previously hard-to-treat groups. Despite these advances, treatment uptake in populations what need HCV treatment the most remains low. There are multiple individual-level and structural barriers that need to be addressed in order to improve effectiveness of treatment at a population level. Novel strategies that integrate HCV prevention and treatment are needed to truly impact the burden of HCV at the population level.

Dr. Mel Krajden, BCCDC, Vancouver, Canada

Biography

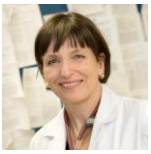
Mel Krajden MD, FRCPC oversees the Hepatitis Section of Clinical Prevention Services and is the Associate Medical Director of the Public Health Microbiology and Reference Laboratory at the BC Centre for Disease Control. He is also a Professor in the Dept. of Pathology and Laboratory Medicine at the University of British Columbia. His clinical research involves integration of hepatitis prevention and care. His laboratory research involves the application of molecular techniques to: diagnose viruses; assess correlates between infection and clinical disease; monitor antiviral efficacy; and track microbial infections for epidemiological purposes. He has extensive clinical trials expertise and serves as a laboratory coordinator for a number of industry sponsored clinical trials. He receives CIHR funding in the fields of HPV, HIV and HCV.

Abstract

The Potential Impact of New Hepatitis C Treatments: How Should Public Health Respond?

Background: Hepatitis C virus (HCV) is becoming increasingly virologically curable. However, HCV related healthcare costs and mortality result from both risk activities that lead to HCV acquisition as well as co-morbid conditions and infection related sequelae.
Methods: We used anonymized linkage of laboratory test results, Public Health Information Surveillance and administrative data to track population level HCV disease trends in British Columbia (BC).
Results: As of Dec 31, 2011, 64,370 anti-HCV positive individuals including 7,405 seroconverters were identified in BC since 1992. Seroconversion timeframes were: 30%, 21% and 49% within 12, 24 and > 24 months respectively. Although age specific anti-HCV testing has remained similar for 1992 to 2011, the median age of reactive individuals increased from 35-39 yrs between 1992-1995 to 50-59 yrs between 2006-2011 suggesting a cohort effect. Anonymized data linkage of HIV and HCV surveillance data revealed that of 8,336 HIV positive individuals, 2,330 (48%) were HIV/HCV co-infected and if non-linkable cases were considered, a minimum of 28% were HIV/HCV co-infected. Of reported anti-HCV reactive individuals, 7.6% of HCV infections were HIV co-infected. Of note, in 57% of HCV/HIV co-infections, HCV was diagnosed first, a median of 4.1 yrs prior to HIV diagnosis and in 20% of co-infections HIV was diagnosed first, a median of 1.6 yrs prior to HCV diagnosis. Linkage of laboratory test data to administrative data was used to estimate that in 2005 BC HCV related direct healthcare costs was $136,000,000/yr. Anti-HCV reactive individuals cost about twice as much as anti-HCV negative individuals with costs relating to both activities associated with HCV acquisition, as well as viral related sequelae. Based on Standardized Mortality Ratio analysis, anti-HCV reactive individuals have a 5 to 38 fold higher risk of death compared to the general population with seroconverters having a 38 fold higher risk of drug related deaths.
Conclusions: Linked laboratory, surveillance and administrative data confirm that HCV infection leads to a substantial mortality and health costs that reflect both risk activities as well as viral related sequelae. Disease burden mitigation will therefore require comprehensive prevention programming to reduce the risk of acquisition of HCV and other blood borne infections such as HIV as well as substantially improved access to effective HCV treatment.

Dr. Julie Bruneau, Université de Montréal, Montréal, Canada



Biography

Dr. Julie Bruneau is a clinical researcher and Professor in the Department of Family Medicine at the University of Montreal. She holds a senior clinical research Scholar of the Fonds de Recherche en Santé du Québec. As a clinician, she is recognized as a leader in the development of addiction medicine in Canada. She was a founding member of the Canadian Society of Addiction Medicine, and implemented the largest University-based Addiction Medical Facility in Eastern Canada.
For the past twenty years, she has conducted epidemiological research among active injecting drug users (IDU), and published her work in high-impact journals. Her research accomplishments have significantly contributed to a better understanding of the dynamics of HIV and HCV transmission among IDUs. Her work on the relation between syringe access and HIV transmission, albeit controversial at times, directly influenced changes in prevention strategies to better address injector needs nationally and around the world. Recently, she has expanded her research program to examine the impact of various approaches including treatment and motivational interviewing, on the behaviours and quality of life of active injection drug users (IDU).

Abstract

Illicit prescription opioids and HCV transmission among injection drug users

The growing availability of opioid analgesics worldwide has been accompanied by an increase of prescription opioid (PO) misuse among various populations of street-based drug users. According to recent ethnographic work, the PO injection process entails various steps, yielding opportunities for HCV transmission. There is limited evidence available on the independent role of PO injection as a determinant of HCV infection and whether opiate replacement treatment (ORT) can mitigate this epidemic. The objective of this presentation is to assess the association between PO injection and HCV seroconversion, and to examine the potential impact of ORT on the risk of infection among IDUs. A prospective cohort study of HCV-negative IDU having injected in the past six months was carried out between 2004 and 2009 in Montreal, Canada. Of the 246 participants, 83 seroconverted to HCV, for an incidence rate of 18 per 100 person-years. In multivariate analysis, PO injectors were 1.9 times more likely to become infected. ORT (mostly with methadone) was not associated with a reduction of HCV transmission. Other independent predictors of HCV incidence include cocaine injection, recent incarceration, and frequency of injection. Thus, PO injection appears to be an important risk factor for HCV acquisition among IDUs. ORT did not reduce HCV transmission in this population.

Clinical Sciences

Prof. Jean-Michel Pawlotsky, Université Paris Est, Créteil, France

Biography

Dr. Jean-Michel PAWLOTSKY is Professor of Medicine at the University of Paris-Est. He is the Director of the National Reference Center for Viral Hepatitis B, C and Delta and of the Department of Virology at the Henri Mondor University Hospital in Créteil, France, and Director of the Department of Molecular Virology and Immunology at the "Institut Mondor de Recherche Biomédicale" (INSERM U955). He focuses on teaching, diagnosis and research in virology, primarily hepatitis viruses. Dr. Pawlotsky earned his medical degree in Hepatology and Gastroenterology in 1992. In addition, he earned a Thesis in molecular virology from the University of Paris, France, and he is a graduate in virology from the Pasteur Institute in Paris and microbiology from the University of Paris. Dr. Pawlotsky is active in numerous professional societies, and has been acting as the Secretary General of the European Association for the Study of the Liver (EASL) between 2005 and 2009. He is a member of the Scientific College and President of Scientific Commission 4 (CSS4) and Concerted Action 33 (AC33) of the French National Agency for AIDS and Viral Hepatitis Research (ANRS). Dr. Pawlotsky has been an Associate Editor of *Hepatology*, the official journal of the American Association for the Study of Liver Diseases (AASLD), between 2001 and 2006, and is currently an Associate Editor of *Gastroenterology*, the official journal of the American Gastroenterological Association (AGA). He is a member of the Editorial Board of the *Journal of Hepatology*, *Therapeutic Advances in Gastroenterology,* and *European Gastroenterology and Hepatology Review.* Dr. Pawlotsky's noted career contributions include the publication of over 350 articles and book chapters in his areas of expertise.

Abstract

Direct acting antivirals for HCV and resistance

Current treatment of chronic hepatitis C virus infection is based on the combination of pegylated interferon-α and ribavirin. The recent development of direct-acting antiviral molecules active on hepatitis C virus, together with *in vitro* and *in vivo* studies showing that these drugs may lead to the selection of resistant viruses if administered alone, has raised concerns that resistance may undermine therapy based on direct acting antivirals. A new standard-of-care treatment is now available for both treatment-naïve and -experienced patients infected with hepatitis C virus genotype 1, based on a triple combination of pegylated interferon-α, ribavirin and a protease inhibitor, either telaprevir or boceprevir. With this therapy, most failures to eradicate infection in treatment-adherent patients are due to an inadequate response to pegylated interferon-α and ribavirin, in the context of a low genetic barrier to resistance of first-generation protease inhibitors. The lecture will review patterns of resistance to hepatitis C virus direct acting antiviral drugs in development, the mechanisms underlying treatment failure when these drugs are combined with pegylated interferon-α and ribavirin, the consequences of treatment failure, and possible means of optimizing therapies using direct acting antivirals in the future.

Dr. Rob Myers, University of Calgary, Calgary, Canada

Biography

Dr. Rob Myers is Associate Professor of Medicine and Director of the Viral Hepatitis Clinic at the University of Calgary. He received his Internal Medicine training at the University of Western Ontario, Gastroenterology fellowship at the University of Calgary, and Hepatology research fellowship at the Université de Paris VI in Paris, France. In 2008, Dr. Myers was awarded his MSc in Epidemiology from the University of Calgary for his thesis entitled "The Epidemiology and Natural History of Primary Biliary Cirrhosis in the Calgary Health Region: A Population-Based Study."

His research interests include the noninvasive assessment of liver fibrosis, epidemiologic and health outcomes research in various liver diseases, and the investigation of novel therapies for autoimmune and viral liver diseases. He is funded by the Canadian Institutes for Health Research (CIHR) and the Alberta Heritage Foundation for Medical Research, and is actively involved in investigator-initiated and industry-sponsored clinical trials. Dr. Myers is the past Chair of the Education Committee of the Canadian Association for the Study of the Liver (CASL) and a member of the National Education Advisory Committee of the Canadian Liver Foundation.

Abstract

Non-invasive Methods for the Assessment of HCV-Related Liver Fibrosis: Challenges and Controversies

The non-invasive assessment of HCV-related liver fibrosis using serum markers and transient elastography has entered mainstream clinical practice. Nevertheless, methodological challenges remain for research in this field and controversy exists regarding the optimal approach to incorporating results into patient management. In this presentation, I will discuss challenges to the practical application of these tests including measurement variability and the difficulty of identifying clinically important changes in measurements, the influence of extraneous factors, and disease specificity. In addition, the use of tests in isolation versus combination algorithms will be reviewed.

Dr. Brian Conway, University of British Columbia, Vancouver, Canada

Biography

Brian Conway, M.D. is a full-time Professor at the University of British Columbia, Department of Anesthesiology, Pharmacology and Therapeutics. He is Coordinator of the Downtown Infectious Diseases Clinic in Vancouver and Infectious Diseases Consultant with the Vancouver Coastal Health Authority, which serves the inner city population on the infamous "Downtown East Side.

Dr. Conway is a member of several professional organizations and has been the Co-chair on the Ministerial Council for the federal initiative on HIV/AIDS since 2008, working with and reporting directly to Health Minister Leona Aglukkak He is the president of the Société Santé en français a federal group charged with the development of health care services for francophone's living in the 9 provinces and 3 territories outside Quebec. He is the past president of the Canadian Association for HIV Research and was the co-chair of the 19th Annual Conference in May 2010 in Saskatoon. He is a proud mentor within the NCRTP group for the past six years.

In addition to supervising several dozen undergraduate, graduate and post-doctoral students over the past decade, Dr. Conway is deeply involved in a range of HIV and HCV-related research and clinical practice efforts. Over the past 5 years, he has played a leadership role in the development of novel strategies for the delivery of care for HIV and HCV in the inner city. These strategies have emphasized the simplification of therapeutic options and integration of medical, addiction and psychological aspects of care. He holds significant research funding to develop a model for the treatment of HCV & HIV infection within a directly observed therapy (DOT) program, focusing on the treatment of intravenous drug users. His programs have received a number of awards for their innovation and success, including the Health Employers Association of British Columbia Award of Excellence in 2008. He was the Francophone of The Year in British Columbia in 2007, for his work in the development of culturally and linguistically optimized systems of care for minority populations.

He is a peer reviewer for 13 medical journals and the primary or senior author of more than 120 peer-reviewed publications. He has been an invited speaker at many international conferences and meetings dealing with HIV and HCV.

Dr. Conway received his medical education at McGill University, from which he graduated in 1982 before completing his internship and residency at Queen Elizabeth Hospital and Royal Victoria Hospital, respectively. He completed a specialty fellowship in infectious diseases at the University of Manitoba in 1988, and a post-doctoral fellowship in HIV/AIDS at Harvard University in 1990. His first staff appointment was as an Assistant Professor at the University of Ottawa in 1990. He moved to the University of British Columbia in 1994, where he is now a tenured Full Professor.

Abstract

Canadian Guidelines for the Treatment of HCV in Injection Drug Users

The prevalence of HCV infection across Canada is highest in injection drug users (58%) [1]. With more than ¾ of new HCV infections today occurring through IDU, the relative importance of this patient population for HCV disease and related public health issues will further increase in the future[1]. Injection drug users patients have a high prevalence of psychiatric disease, have multiple medical problems including HIV and face significant social challenges such as homelessness and lack of supports [2,3]. They are less likely to begin and remain engaged in care without employing a specialized care model[4].

Evidence suggests that properly selected HCV infected IDU patients can achieve SVR at rates comparable to a non IDU population [4-6]. Newly available direct acting antivirals (DAA) decrease duration of therapy and increase cure rates in the most difficult to cure patients, increasing the proportion of suitable candidates for therapy[7]. IDU patients achieving SVR experience re-infection rates lower than expected due to a combination of a change in risk taking behaviours (possibly related to successful engagement in care) and potentially due to acquired immunologic protection [8,9].

Although greater than 80% of HCV infected IDU are interested in therapy for HCV, only 10 to 30% are offered treatment [4, 7, 10, 11]. Based on a high likelihood of success, lower reinfection rates and well established morbidity and viral transmission associated with longstanding viremia, HCV infected IDU patients should be considered for therapy if they meet certain criteria.

We conducted a retrospective analysis of patients receiving HCV treatment through our Pender Community Health Centre. A total of 182 patients were included in this analysis (26 women), with a mean age of 52.2 years. All had a history of IDU (89.2 % by injection), with 40% continuing drug use during HCV treatment. Only 12.4 % patients were cirrhotic, and 30.8 % had genotype 2 or 3 infection, 8 % were co-infected with HIV. Overall, 72.9% completed assigned therapy. Of the total patient population, 19 genotype 1 infected patients (10 % of the total group, 14.8 % of the genotype 1 patients) discontinued due to lack of virologic response. Only 12 patients (6.5 %) had a relapse in IDU leading to non-adherence, and 12 patients (6.5 %) stopped due to drug toxicity. Overall sustained virologic response (intent-to-treat analysis) was 52.2 % for genotype 1 and 73.7 % for genotype 2 or 3 infection.

Taken together, all available data allow us to conclude that HCV infection can be treated successfully and productively in large numbers of IDUs. Discontinuation rates and causes appear to be similar to those previously reported in clinical trials. As will be reflected in 2012 Canadian guidelines for the treatment of HCV infection, IDUs should be considered for therapy under many circumstances, especially within the context of multidisciplinary community-based models for the delivery of health care. This may have a significant impact not only on the health of the treated individuals, but on the community as well as the HCV epidemic as a whole.

Dr. Jenny Heathcote, University of Toronto, Toronto, Canada

Biography

Dr. E.J.L. (Jenny) Heathcote was born in the UK and graduated from the Royal Free Hospital School of Medicine, London, UK in 1968. She was introduced to the field of liver disease immediately as her first position as a resident was with the late Dame Professor Sheila Sherlock – the most famous hepatologist of her time. Following completion of her internal medicine training, Jenny returned to the Sheila Sherlock "empire" where she spent 4 years doing research on the transmission of Hepatitis B - she was awarded her MD thesis in 1976. Jenny emmigrated to the USA and later accepted a staff position in Canada at the University of Toronto where she has remained for 33 years. From scratch she built a clinical program in Hepatology with a particular focus of viral hepatitis (B and later C) and autoimmune liver disease. She is a Senior Scientist in the Toronto Western Research Institute where she is Division Head of "Patient Based Clinical Research".

She has been a Professor at the University of Toronto since 1995, winning the Department of Medicine Clinician Teacher Award in the same year. She was awarded the May Cohen Award by the Canadian Medical Association for her mentoring of trainees in 2003. In this same year she initiated the National Canadian Research Training Program in Hepatitis C (CIHR funded) where she was the PI until the program was refunded in 2009.

She is a recipient of the Queen's Jubilee Gold Medal for her service to hepatology and received the Canadian Liver Foundation Gold Medal in 2004. In that year, she also received the Canadian Liver Foundation Lifetime Achievement Award.

In 2005, she was the recipient of the American Association for the Study of Liver Diseases Distinguished Achievement Award for her sustained scientific contributions to the field of liver disease. In 2006, she received the International Sheila Sherlock Award from the Falk Foundation. In 2008, she was awarded the Department of Medicine, University of Toronto Mentoring Award.

In 2008 she was the only successful Canadian centre applicant to the Hepatitis B clinical consortium funded until 2015 by NIH.

In 2009 she received the Francis Family Chair in Hepatology Research at the University.

She was awarded the EASL International Recognition Award (2010) for her sustained contribution to the knowledge and understanding of liver disease.

She has been funded by the Canadian Institutes of Health Research since 1988.

Over her career, she has published over 350 papers covering almost all topics in non transplant hepatology. She has mentored 64 medical residents during their research electives and has trained 31 fellows in hepatology. She has delivered close to 800 lectures, of which over half have been international.

Abstract

An Academic Career in Medicine (Hepatology)

A successful career in whatever field first requires an ambitious personality but other characteristics are necessary e.g. stamina, vision, determination and perhaps most important the ability to follow through ("staying power"). However, without outside help e.g. mentors, collaborators and support by both colleagues and family, success is unlikely.

Upon reflection I now realize that my high school Math teacher was first to encourage me, followed by Dame Professor Sheila Sherlock – one of the world's leading hepatologists in her time (died 1981) – they both had faith in me. My subsequent time at Stanford University was not what I had wanted but I did learn that I was not cut out to be a laboratory scientist. It is just as important to be able to recognize one's weaknesses as well as strengths. I opted for a career in clinical academic hepatology in a country that provided universal access to healthcare. On my arrival to Canada (1979) I was unknown to most. To become recognized as a reliable authority on any subject one needs the support of colleagues, patients and professional societies – this can only be achieved by hard work, an "outgoing" personality helps. Sustained ability to inspire (students, fellows and colleagues) is necessary to build an academic program. Most of us learn best from our mistakes as long as you recognize and heed them (I can list many of mine)!

Collaboration locally and nationally/internationally is a must – this facilitates student recruitment, funding and good will. Leadership i.e. ability to build something better and maintain excellence by always recruiting individuals who are smarter than you requires good judgement and vision with a great deal of good luck (of which I have had more than my fair share)!

Oral Abstracts – résumés oraux

Biomedical Sciences

Oral presentation at 9h00

Y-BOX-BINDING PROTEIN-1-CONTAINING RIBONUCLEOPARTICLE HIJACKING DURING HEPATITIS C VIRUS LIFE CYCLE

Chatel-Chaix, L., Germain M-A., Motorina, A., Bonneil, E., Thibault, P. and Lamarre, D.

Institut de Recherche en Immunologie et en Cancérologie (IRIC), Centre de Recherche du CHUM (CRCHUM), Hôpital Saint-Luc, Université de Montréal, Québec, Canada, H3T 1J4

The Hepatitis C virus (HCV) NS3/4A protein has several essential roles during the virus life cycle most probably through dynamic interactions with host factors that might represent promising avenues for the development of novel therapeutic strategies. We recently identified Y-box-binding protein-1 (YB-1) as a novel interacting partner of NS3/4A protein which is critical for the life cycle of the JFH1 infectious clone. Importantly, we demonstrated that YB-1 is a positive regulator of HCV RNA replication while it restrains the production of infectious viral particles. Immunofluorescence studies further revealed a drastic HCV-dependent redistribution of YB-1 to the surface of the lipid droplets (LDs), a critical organelle for HCV assembly. This suggests that YB-1 ribonucleoparticles (RNPs) hijacking by HCV capsids is important for the control in time and space of virus particle assembly.

In this study, we took advantage of laser scanning confocal microscopy and co-immunoprecipitation approaches to further characterize YB-1 RNP hijacking during JFH-1 life cycle. First, we demonstrated that the deletion of the cold-shock domain (CSD) of YB-1 severely impairs not only its re-localization at LDs by JFH1 but also its association with NS3 in infected cells. Furthermore, a JFH1 virus strain carrying a mutation in the NS3 protein (Q221L) was reported to produce higher yields of viral particles. This JFH1-expressing NS3 mutant showed a reduced YB-1/NS3 association and failed to recruit YB-1 to LDs as efficiently as wild type JFH1. Altogether, these data strongly suggest that YB-1 is involved in NS3-dependent steps of HCV assembly.

We also demonstrated that core, NS3, YB-1 and DEAD-box protein-3 (DDX3; previously reported to be required for HCV replication) co-localized at the surface of LDs within the same complex. To gain further insight into this HCV-specific RNP, we analyzed the composition of YB-1 interactome in JFH1-expressing cells using mass spectrometry, and showed that several confirmed YB-1 protein partners were also recruited to LDs by assembling capsids. These new co-opted host factors are currently challenged for their potential role during HCV assembly.

Overall, our data support a model in which HCV, via an NS3/YB-1 association, hijacks RNPs containing YB-1, DDX3 and other host factors that regulate the NS3-dependent steps of HCV particle assembly. We propose that the "over-assembling" properties of the NS3 Q221L mutant may be due to its capacity to by-pass a negative regulation imposed by YB-1-containing RNPs for particle production.

Funding source: CIHR-MOP-1150858.

Oral presentation at 9h15

THE ROLES OF FITNESS AND THE GENETIC BARRIER IN THE CONTEXT OF SYNONYMOUS NUCLEOTIDE CHANGES DURING HCV REPLICATION

Robert A. Kozak[1], Megan Powdrill[2], Heidi Morris[4], Rodney Russell[4], Arnim Pause[3] and Matthias Götte[1,2]

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[4]Division of BioMedical Sciences, Memorial University, St John's, Newfoundland, A2H 6P9

Background: The HCV RNA-dependent RNA polymerase, NS5B, is error-prone and lacks an intrinsic proof-reading mechanism. This results in base pair mismatches that can be seen as precursors for mutations that are generated during replication. Mutations that confer resistance to antiviral drugs eventually emerge and dominate the viral population. We have recently demonstrated that HCV replication is characterized by a strong mutational bias for transitions over transversions (Powdrill et al PNAS, 2011). These data suggest that mutations that arise through the transition pathway are more easily selected. However, sub-optimal codon usage at the same position can also compromise viral fitness. Thus, we hypothesize the existence of a balance between the genetic barrier and replicative fitness that determines the outcome of the selection process.
Purpose: We hypothesize that there are two genetic pathways by which an amino acid change can occur. An "easy" pathway, (i.e. through transitions) and more difficult pathways, (i.e. through transversions). We intend to test whether this principle holds true for a novel mutation reverting back to a wild-type sequence, and evaluate effects on replicative fitness.
Method: We selected arginine residues present in the thumb domain of NS5B that are conserved across multiple subtype 1b strains. These residues were mutated to a tryptophan, as reversions can occur through either a transition (tryptophan TGG $\rightarrow$ arginine CGG), a transversion (tryptophan TGG$\rightarrow$ arginine AGG), or multiple changes. Whether fitness is an additional parameter for the selection of synonymous changes remains to be seen. Site-directed mutagenesis was performed in the Con1 (genotype 1b) replicon system, and replication in Huh-7 Lunet cells was evaluated using Taqman probes. To compliment these studies all variants of the original arginine were generated to evaluate both fitness, and whether non-conserved variants would revert back to wild-type.
Results: We identified reversions at several positions that were included in initial screening efforts Once HCV replication was unambiguously shown, total RNA was isolated for sequencing to determine the genetic change(s). End-point experiments revealed that several mutants reverted to the wild-type codon even if this required multiple changes, or the transversion pathway. Fitness experiments show that non-wild type codon variants of the natural amino acid were less fit than the wild-type codon. Additionally, when the arginine codon was mutated to a variant not present in the wild type replicon (eg. CGC$\rightarrow$ CGG), it was observed to revert back to the wild-type codon in cell culture.
Conclusion(s): These studies show that there is a dynamic interplay between genetic barrier and fitness that can both determine the outcome of a selection process, even for synonymous changes. In our studies, it appears that in the C-terminal region of the thumb domain of NS5b, replicative capacity was favored over the genetic barrier. Additionally residues outside of the thumb domain will be investigated to determine whether the preference for codon conservation is exclusive to this region.
Funding Sources: Canadian Institutes of Health Research (CIHR), National CIHR Research Training Program in Hepatitis C (NCRTP-HepC).

Oral presentation at 9h45

MODULATION OF HCV SPECIFIC CD8 T CELLS EXHAUSTION AND SURVIVAL BY IL-21 DURING ACUTE HEPATITIS C

Hassen Kared[1], Nathalie Bédard[1], Thomas Fabre[1], Julie Bruneau[1,2] and Naglaa H. Shoukry[1,3]

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Background: CD4 helper T cells are essential for spontaneous clearance of hepatitis C virus (HCV). Their failure during the early acute phase of HCV was associated with viral recurrence and a chronic disease course but the underlying mechanism(s) are not well understood. CD4 T cell help is crucial to sustain survival, expansion and cytotoxic activity of CD8 T cells. Recent studies in the LCMV and HIV models have proposed that IL-21 could be a prime candidate for mediating CD4 help.

Method: In the present study, we investigated the role of IL-21 during acute HCV infection with either spontaneous resolution (n=19) or persistent viremia (n=19). Longitudinal analysis demonstrated an increase in plasma IL-21 levels during the late acute phase in spontaneous resolver patients.

Result(s): (i) we have identified a subset of Th17 cells characterized by the co-expression of CCR6, CD26 and CD161 as the cellular source of IL-21. This subset was increased in frequency in the peripheral blood of patients who spontaneously resolved acute HCV.

(ii) Virus-specific CD4 T cells displayed a strong Th1 (IFN-γ and TNF-α) and Th17 (IL-17 and IL-21) cytokines profile in spontaneous resolver patients. In contrast, limited Th17 antigen specific responses were observed in HCV chronically infected patients. Plasma IL-21 correlated with frequency of HCV-specific CD8 T cells as measured by MHC class I tetramers.

(iii) Exogenous IL-21 rescued proliferation of HCV-specific T cells and prevented CTL apoptosis induced by interaction of the inhibitory receptor Tim-3 with its ligand galectin-9 in chronic HCV infection. This underscores the role of IL-21 as a helper cytokine during acute HCV.

(iv) To determine the mechanism(s) that regulate IL-21 production during chronically evolving HCV, we evaluated the cross regulation between Th17 and regulatory T cells (Tregs). We observed an increase in CD39+ Treg cells expressing galectin-9. Furthermore, the capacity to produce IL-17 and IL-21 was enhanced upon blockade of the inhibitory receptors Tim-3.

Conclusion(s): Our results suggest a central role for IL-21 producing Th17 cells in modulating T cell survival and function during acute HCV. We propose that failure of CD4 T cell help during chronic HCV is mediated by imbalance and cross-regulation between Th17 and Tregs through the Tim-3/galectin-9 pathway.

Funding source: IRSC, Réseau SIDA/ MI FRSQ, University of Montréal.

Oral presentation at 10h00

DOES A VACCINE DERIVED FROM A SINGLE HCV STRAIN ELICIT BROAD CROSS-NEUTRALIZING ANTIBODIES IN HUMANS?

John Law, Jason Wong, Chao Chen, Darren Hockman, Sharon Frey*, Robert Belshe*, Takaji Wakita**, Jens Bukh#, Charles Rice^, Steve Coates^^, Sergio Abrignani^^^ & Michael Houghton Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Canada

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Background: Hepatitis C virus (HCV) is a global health concern. Our understanding of the HCV biology has been rapidly expanding and discoveries of new antivirals are showing great promise. However, an effective vaccine preventing acute or chronic HCV infection remains elusive. Successful vaccines developed to date protect largely through the induction of neutralizing antibodies in the case of viral pathogens and through theinduction of bacteriocidal antibodies in the case of bacterial pathogens. Furthermore, cross-neutralizing HCV antibodies have been shown to be prophylactic in animal models.
However, a key challenge in the field of HCV vaccine research relates to the ability of vaccines to elicit antibodies that can cross-neutralize multiple clades of these highly heterogeneous viruses since much data has been presented suggesting that neutralizing antibodies against HCV may be narrowly restricted or only isolate-specific.
Purpose: To Test for cross neutralization activities against various HCV genotypes in sera of phase I clinical trial volunteers who were immunized with recombinant HCV glycoproteins (gpE1/gpE2) derived from a single original genotype 1a HCV1 stain.
Method: Sera samples have been assayed for the presence of antibodies that can neutralize infectious HCVcc derived from Huh-7.5 human hepatoma cell cultures containing glycoproteins derived from heterologous 1a, 1b, 2a and 2b HCV strains.
Results: We found that the majority of vaccine recipients elicited antibodies that could neutralize heterologous genotype 1a & 1b which represent the most common clades around the world. In addition, most sera contained antibodies that could also partially neutralize genotypes 2a and 2b. **Conclusions:** Our results suggest a vaccine derived from a single strain of HCV can elicit broadly cross-neutralizing antibodies. We are currently testing cross-neutralization activity of these antibodies to other genotypes including genotypes 3 through 7. We hypothesize antibodies with broad cross-neutralization activity recognize conserved epitope(s) on the glycoproteins to block infection. Identification of these crossneutralizing epitopes and their mode of action are critical for effective vaccine design.
Funding Source: Canada Excellence Research Chair in Virology (Michael Houghton).

Behavioral Sciences

Oral presentation at 11h45

NOTHING FOR US, WITHOUT US: THE MEANINGFUL INVOLVEMENT OF CLIENTS IN HCV TREATMENT AND PREVENTION

Kate Mason, Research Coordinator South Riverdale Community Health Centre. Zoe Dodd Hep C Program Coordinator South Riverdale Community Health Centre

Background: The East Toronto Hepatitis C Program (ETHCP) is a community-based, interdisciplinary model of Hepatitis C treatment, group support and education for homeless and low-income individuals who may also use substances and/or have serious mental health issues and/or HIV co-infection. The program began in 2006 and is a partnership between three community health centres, with specialist support from nearby hospitals.
Purpose: This presentation will focus the role of peer support and on the meaningful participation of clients in the development of this innovative model of HCV treatment. It will explain how the integrated peer support, training and advisory components of the ETHCP has improved Hep C treatment uptake and care for the target population, as well as their indirect effects on client well-being and Hep C prevention in the larger community.
Method: At each program site, HCV treatment is centred on a weekly psycho-educational group for people living with HCV and/or in various stages of pre/post treatment. Groups are closed and limited to approximately 20 people. Group members access HCV treatment providers during and before/after the 2 hour group meeting. A Patient Advisory Board made up of clients from each program site meets monthly to provide input and feedback on program development, implementation and research. A peer training program was initiated in the fall and has trained 10 clients to become peer support workers. These peer roles include outreach, appointment accompaniment, and post-treatment support.
This presentation will discuss how peer support and input are integrated at various stages and levels of the ETHCP. The role and accomplishments of the Patient Advisory Board will be outlined. A member of the advisory board will share his story and experience of the program.
Results: The meaningful involvement of people with lived experience of HCV has been critical to the success of the ETHCP with important impacts for clients, health care providers and the larger community.The ETHCP group support model has improved access to HCV treatment for illicit substance users and other marginalized individuals, with SVR rates that are comparable to clinical trials. The program has empowered clients to take control of their health and has created a network of peer educators, advocates and mentors. Participation in the Patient Advisory Board has positive impacts on self-esteem and sense of community-belonging. The involvement of clients in program development helps to ensure high quality care, relevancy and accountability. Findings from a retrospective chart review of 129 clients and a recent program evaluation will be presented.
Conclusions: The ETHCP is a model of care that improves access to HCV treatment by addressing many of the complex needs and barriers faced by people living with Hep C. The meaningful involvement of people living with HCV has been vital to the integrity and ongoing success of this program.

Epidemiology and Public Health

Oral presentation at 14h00

FEMALE SEX AND VARIATIONS IN IL28B ARE INDEPENDENTLY ASSOCIATED WITH SPONTANEOUS CLEARANCE OF ACUTE HCV INFECTION

Jason Grebely[1], Greg J. Dore[1], Maarten Schim van der Loeff[2], Tom Rice[3], Andrea L. Cox[4], Julie Bruneau[5], Arthur Y. Kim[6], Jacob George[7], Lisa Maher[1], Andrew R. Lloyd[8], Margaret Hellard[9], Kim Page[3] and Maria Prins[2], on behalf of the InC3 Collaborative Group

[1]The Kirby Institute for infection and immunity in society, University of New South Wales, Sydney, NSW, Australia, [2]GGD Public Health Service of Amsterdam, Amsterdam, The Netherlands, [3]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA. [4]Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD, USA, [5]CRCHUM, Université de Montréal, Montreal, QC, Canada, [6]Harvard Medical School, Boston, MA, USA, [7]Storr Liver Unit, Westmead Millennium Institute, University of Sydney, Sydney, Australia, [8]School of Medical Sciences, University of New South Wales, Sydney, NSW, [9]Burnet Institute, Melbourne, VIC, Australia.

Background: Studies have demonstrated that variations in the interleukin-28 (*IL28B*) gene region are associated with spontaneous HCV clearance. However, many of these studies have been in cross-sectional cohorts comparing HCV RNA negative and positive individuals for whom the duration of infection is unknown.
Purpose: We evaluated factors associated with spontaneous clearance (including *IL28B* genotype) during acute HCV infection.
Methods: The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC^3) is a collaboration of nine cohort studies from Australia, Europe and North America. Data on HCV seroconversion among IDUs enrolled into these cohorts between 1986 and 2009 were included. Inclusion criteria were: history of IDU, documented anti-HCV seroconversion within a two-year period and minimum of two therapy-naïve HCV RNA assessments following the estimated date of seroconversion. We estimated the proportion with spontaneous clearance during the first two years following infection and identified factors associated with spontaneous clearance using logistic regression.
Results: Among 517 with incident HCV infection (173 females), HCV genotype (G) prevalence was: G1, 49%; G2, 6%; G3, 30%; G4, 1%; G6, 1%; mixed genotypes, 2% and unknown genotypes, 11%. The prevalence of IL28B genotypes was 50% CC, 37% CT and 12% TT (n=406). Spontaneous clearance was observed in 26% (95% confidence interval: 22%, 31%; 136 of 517). Spontaneous clearance was higher among females (38% vs. 21% males, P<0.001) and among those with favorable CC IL28B genotype (37% vs. 24% CT/TT, P=0.005). Among females with IL28B (n=131), the proportion with spontaneous clearance was 58% in those with favorable CC genotypes as compared to 27% in those with unfavorable CT/TT genotypes (P=0.002). In unadjusted analysis, factors associated with spontaneous clearance included female sex, Aboriginal ethnicity, no recent IDU and favorable CC *IL28B* genotype (vs. CT/TT). In adjusted analysis, female sex [adjusted (AOR) 2.66; 95% CI: 1.69, 4.18; P<0.001] and favorable CC *IL28B* genotype (vs. CT/TT: AOR 2.03; 95% CI: 1.30, 3.19; P=0.002) were independently associated with spontaneous clearance. The interaction between female sex and IL28B was assessed, but was not statistically significant.
Conclusions: Female sex and *IL28B* genotype are independently associated with spontaneous clearance during acute HCV. Delayed therapeutic intervention during acute HCV infection could be recommended for females with favorable *IL28B* genotypes to allow time for spontaneous clearance.

Oral presentation at 14h15

LONG-TERM DIRECT HEALTH CARE COSTS ATTRIBUTABLE TO HEPATOCELLULAR CARCINOMA; A POPULATION-BASED STUDY

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Background: Canadian estimates suggest a trend towards substantial increases in hepatocellular carcinoma (HCC) incidence in recent decades, essentially as a result of an underlying cohort effect linked to an increase in the incidence of hepatitis C.
Purpose: To estimate phase-specific health care costs attributable to HCC, 5-year net costs of HCC care and determine predictors of health care costs associated with HCC across disease phases.
Methods: We used the Ontario Cancer Registry to identify persons principally diagnosed with HCC between January 1, 2002 and December 31, 2008. Patients were followed from the day of diagnosis up until death or until to the end of the study period, with additional 12 months of observation to capture deaths. We used joinpoint regression analysis to allocate patients' observation time to phases: Initial; Continuing Care; and Pre-death. In Cohort 1, all cases were matched 1:1 with controls to estimate costs for the Initial and Continuing Phases. In Cohort 2, cases who died as a result of HCC were matched 1:1 to controls who died from other causes to estimate costs for the Pre-death Phase. For each cohort, we selected the closest non-HCC control from the Registered Persons Database that met the following criteria: age +/- 10 years at the index date; within 30 days of the index year; Charlson-Deyo Comorbidity Index category (0, 1, 2, > 3, no hospital record), and a propensity score within a caliper width of 0.2 standard deviation. We captured costs for outpatient services, emergency visits, same day surgery, acute inpatient care, medications, home care, continuing care, and long-term care. Direct health care costs (per 30 patient-days) attributable to HCC for each disease phase were estimated using Generalized Estimating Equation models. Estimates of mean 30-day net costs of care for HCC patients in each disease phase were applied to monthly survival probabilities after diagnosis accounting for competing risks to calculate 5-year net costs of care. Generalized linear regression was used to determine predictors of health care costs associated with HCC.
Results: Overall, 2,423 HCC cases were identified. Over the period, the number of new HCC cases increased, and although the proportion of those aged 80 and above increased, Comorbidity Index remained stable. Treatment with liver transplant, radiofrequency ablation, or transarterial chemoembolization in the year after HCC diagnosis increased over time. Mean (95% confidence intervals, CI) total costs (per 30 days in 2010 Canadian dollars, undiscounted) attributable to HCC were $4,898 ($4,467-$5,330) in the Initial Phase, $2,615 ($2,054-$3,177) in the Continuing Care Phase, and $1,518 ($477-$2,559) in the Pre-death Phase. Estimates of the mean (95% CI) 5-year net costs of HCC care were $67,831 ($49,565-$86,097) (undiscounted), $47,238 ($34,888-$59,589) (3% discount), and $44,284 ($32,900-$55,667) (5% discount). Comorbidity was a significant predictor in all phases, with a significant trend towards higher costs in patients with greater comorbidity.
Conclusions: Our results suggest that the costs of care attributable to HCC are substantial and vary by disease phase. The initial phase costs the most out of the three phases.
Implications: These results provide useful information for planning of cancer control and policy decision-making to mitigate the burden of hepatitis C in Canada.
References: 1. Pocobelli G, Cook LS, Brant R, et al. Hepatocellular carcinoma incidence trends in Canada: analysis by birth cohort and period of diagnosis. Liver Int 2008; 28:1272-1279.
2. Dyer Z, Peltekian K, van Zanten SV. Review article: the changing epidemiology of hepatocellular carcinoma in Canada. Aliment Pharmacol Ther 2005; 22:17-22.

Funding source: This study was supported through provision of data by the Institute for Clinical Evaluative Sciences (ICES) and Cancer Care Ontario (CCO) and through funding support to ICES from an annual grant by the Ministry of Health and Long-Term Care (MOHLTC). The opinions, results and conclusions reported in this paper are those of the authors. No endorsement by ICES, CCO or the MOHLTC is intended or should be inferred.

Clinical Sciences

Oral presentation at 16h00

DONOR INTERLEUKIN-28B POLYMORPHISMS ARE ASSOCIATED WITH HEPATIC FIBROSIS AND INFLAMMATORY MOLECULAR CHANGES FOLLOWING LIVER TRANSPLANTATION IN PATIENTS WITH HEPATITIS C INFECTION

Brad Thomas, Stephen Osasan, Aldo Montano-Loza, Luis Hidalgo, Phil Halloran, Lorne Tyrell and Andrew Mason, Banu Sis

Background: Recurrence of HCV after transplantation is universal and response rates to standard antiviral therapy are poor. Recently, genetic variation in the interleukin-28B (IL28B) gene, encoding interferon λ3, has been associated with treatment-induced viral clearance in nontransplant and transplant patients. Histological and molecular consequences of this genetic variation are unknown. We aimed to gain further insight into the effects of IL28B SNP status on hepatic tissue.
Methods: Genotyping of IL28B rs12979860 and rs8099917 was perfomed by TagMan real-time PCR using PBMCs in 16 HCV-infected liver transplant recipients and their donors. We related IL28B genotypes with histological activity, fibrosis stages, and gene expression profiles by Affymetrix genechip in 25 liver transplant protocol or clinically indicated biopsies. None of the patients received antiviral therapy.
Results: The frequencies of donor IL28B genotypes (available in 13 of 16 liver transplants) at rs12979860 were 5 C/C, 6 C/T, and 2 T/T, whereas, at rs8099917 we had 8 T/T, 5 T/G, and 0 G/G. Recipient genotypes at rs12979860 were 8 C/C, 7 C/T, and 1 T/T, whereas, at rs8099917 we had 11 T/T, 4 T/G, and 1 G/G. The donor rs12979860 C/C genotype was related with higher histological hepatic fibrosis stages, portal inflammation, bile duct injury, and venulitis when compared to C/T and T/T genotypes (Figure 1). The donor rs8099917 T/T genotype was related with high fibrosis stages and more venulitis. Furthermore, rs12979860 C/C genotype was associated with increased intrahepatic expression of T cell and plasma cell (immunoglobulin) transcripts and decreased parenchymal transcripts, and rs8099917 T/T genotype was related with increased plasma cell transcripts ($p<0.05$). In contrast, recipient IL28B genotypes were not related to histology activity, fibrosis stages or expression of transcript sets. HCV viral loads were not different among IL28B genotypes.
Conclusions: Donor IL28B genotypes are associated with more severe histological recurrence of HCV infection. Our results suggest a dominant impact of the donor rather than the recipient IL28B polymorphisms on the natural course of HCV liver transplant reinfection.

Oral presentation at 16h15

PEGYLATED INTERFERON-ASSOCIATED RETINOPATHY IS FREQUENT IN HCV PATIENTS WITH HYPERTENSION AND JUSTIFIES OPHTHALMOLOGIC SCREENING

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Background: Treatment with pegylated interferon alpha (PegIFNα) and ribavirin is still regarded as the standard of care for chronic hepatitis C. Even though two direct antiviral agents (DAAs) have been recently approved for the treatment of hepatitis C due to HCV genotype 1, PegIFNα plus ribavirin will remain the backbone in any future combinations for several years. Thus, the clinician has still to manage side effects related to treatment with PegIFNα and ribavirin. Retinopathy has been occasionally described in case report series but prospective, longitudinal data are lacking.
Purpose: This is the first dedicated, prospective study aimed to investigate the frequency and clinical significance of retinopathy during therapy with PegIFNa and ribavirin.
Method: 97 consecutive HCV patients were enrolled. 54 (55.7%) and 43 (44.3%) patients were treated with PegIFNa 2a and PegIFNa 2b, respectively. Ophthalmologic examination was performed before therapy (baseline), at 3 and 6 months (3T and 6T, respectively) of therapy and 3 months after the end of therapy (3ET). All patients underwent the baseline and 3T examination, 90.7% underwent 6T and 3ET examination.
Result(s): Overall, 30.9% of patients developed retinopathy, as defined by the presence of cotton wool spots and/or retinal haemorrhages. Variables significantly associated with retinopathy during treatment were age ($p=0.004$), metabolic syndrome ($p=0.05$), hypertension ($p<0.0001$), cryoglobulinaemia ($p=0.05$) and preexisting intraocular lesions at baseline ($p=0.05$). By multivariate analysis, the only variable independently associated with PegIFNa-associated retinopathy was hypertension (HR=4.99, 95% CI 2.29-10.89). The frequency of retinopathy at baseline and at all treatment time-points was significantly higher in hypertensive patients vs. those without hypertension (18.5% vs. 5.7% at baseline, $p=0.05$; 48.1% vs. 15.7% at 3T, $p=0.0009$; 76.0% vs. 16.2% at 6T, $p<0.0001$; 32.0% vs. 6.2%, $p=0.0005$ at 3ET). In one (1.1%) hypertensive patient, who developed bilateral branch retinal vein occlusion at 6T, the therapy was discontinued.
Conclusion(s): Retinopathy is frequent during treatment with PegIFNα and ribavirin, especially in hypertensive patients, who may develop serious complications. Screening for PegIFNα-associated retinopathy should be recommended for HCV patients with hypertension. Since all cases of retinopathy developed by 6T, we suggest that HCV patients with hypertension undergo an ophthalmologic examination at baseline, 3T and 6T and continue after treatment cessation if intraocular lesions are documented at any of the previous time points
Funding source (f): None.

Posters-Affiches

Biomedical Sciences

Poster number: 100

VIRAL DETERMINANTS OF THE OUTCOME OF INTERFERON THERAPY AGAINST HEPATITIS C VIRUS

Mohamed S. Abdel-Hakeem[1,2], Thomas Fabre[1,2], Jean-François Gélinas[1], Mohamed El-Far[1,2], Donald Murphy[5], Julie Bruneau[1,4], Bernard Willems[1,3] and Naglaa H. Shoukry[1,3]

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Background & Aims: We have previously demonstrated that early initiation of interferon-alpha (IFN-α) therapy can rescue poly-functional HCV-specific CD8+ T cells (1). In contrast, no HCV-specific immune responses were detected when therapy was initiated late during chronic HCV (2). This leaves the virus as the major determining factor for the differential responses to therapy during the chronic phase. One potential mechanism of HCV-resistance to therapy is blocking the IFN signalling pathway. The direct inhibitory effects of the HCV proteins, core and NS5A, on IFN signalling have already been shown using reference laboratory HCV strains (3). However, the effect of core and NS5A isolated from patients with differential responses to IFN therapy remains to be examined. Our aim is to identify HCV sequences from clinical isolates that impair IFN signalling and define mechanisms underlying this impairment.
Methods: The core regions are sequenced from isolates of patients who received IFN therapy during the chronic phase, with differential responses. The different sequences are expressed in hepatoma cell lines and their capacities to impair IFN signalling are being measured. The effects of the different sequences on HCV fitness will be measured using the JFH1 culture system (4).
Results & Conclusions: We have amplified and sequenced the core region from patients infected with HCV genotype 1 (n=20), with differential responses to IFN therapy. No significant differences were observed amongst genotype 1a sequences from responders versus non-responders or relapsers. In contrast, genotype 1b sequences from one non-responder displayed specific amino acid (aa) substitutions; Q70R and M91L in 80% and 90% of the molecular clones before therapy, respectively. Similar aa substitutions have been associated with resistance to therapy in several Japanese cohorts (5). The resistant substitutions were selected-for during therapy and became dominant at the end of the therapy period (100% Q70 and M91). In addition, non-responder sequences showed a higher degree of quasi-species diversity pre-therapy compared to responders and relapsers. The IL28B genotype is currently being analysed for all patients. Sequencing more patients is ongoing to determine whether specific aa substitutions can serve as prognostic markers for response to IFN therapy in the North American population, combined with IL28B genotyping. Whether the identified sequences have differential capacities to impair IFN signalling and affect HCV fitness, remains to be determined.
References:
1- Badr G. et al., J.Virol. 2008.
2- Abdel-Hakeem MS et al., J. Virol., 2010.
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4- Wakita T. et al., Nat. Med., 2005.
5- Akuta N. et al., Intervirology, 2005.
* This work is supported by grants from the Canadian Institutes for Health Research (CIHR) (MOP-106468) and Le Fonds de recherche du Québec - Santé (FRQS) (FRQS-12428). Mohamed Abdel–Hakeem is the recipient of doctoral fellowships from the CIHR and the National CIHR Research Training Program on Hepatitis C (NCRTP-Hep C) and Université de Montréal.

Poster number: 101

FRET-BASED ASSAY TO MONITOR BINDING OF HCV NS3 HELICASE TO ITS NUCLEIC ACID SUBSTRATE

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Background: The C-terminal domain of non-structural protein 3 (NS3) encodes a helicase which is required for viral replication. The helicase domain (NS3h) binds both single-stranded RNA and DNA, and travels in a 3' to 5' direction to promote unwinding.
Purpose: To develop a plate-based Fluorescence Resonance Energy Transfer (FRET) assay to detect binding of the helicase to nucleic acid substrates. The assay is designed to provide information on the specific location of the NS3h enzyme on the substrate.
Methods: Purified NS3h containing 14 native cysteine residues was non-specifically fluorescently labeled with Cy5-maleimide under selective conditions. The fluorescently labeled enzyme was then used in combination with various Cy3 fluorescently labeled nucleic acid substrates in order to detect binding by FRET. Using a DNA oligonucleotide 5' labeled with the Cy3 donor dye, partially double stranded hybrids were formed by annealing shorter DNA oligonucleotides so as to have different hybrids with increasing length of the 3' single stranded helicase loading region.
Results: As expected, when the labeled enzyme is incubated with partially double-stranded substrates with increasing lengths of the single stranded loading region the FRET signal increases as the enzyme can bind on average closer to the fluorophore at the 5' end of the loading strand. By adding increasing concentrations of unlabelled NS3h the FRET value decreases, as the unlabelled enzyme competes with the labeled enzyme for binding to the substrate, providing further proof-of-principle for the assay. Separately, upon addition of ATP to initiate unwinding catalyzed by the helicase, the FRET value also decreases as the enzyme unwinds the double stranded region and dissociates from the nucleic acid. This effect is enhanced upon addition of a trap for the unwound labeled strand of the substrate. By redesigning the partially double-stranded substrates so that the fluorophore is at the ss/ds junction, with increasing lengths of single stranded loading region there is no decrease in the FRET signal, indicating that the enzyme sits at the ss/ds junction, suggesting ATP is only required to continue forwards through the double stranded region.
Conclusion: The FRET-based assay has the potential to monitor distinct steps of the unwinding process. It can be translated in a high-throughput format, which provides a novel tool for the discovery of small molecules that interfere with the NS3h enzyme.
Funding Sources: Canadian Institutes of Health Research (CIHR), Fonds de Recherche du Québec Nature et Technologies (FQRNT), The CIHR Strategic Training Initiative in Chemical Biology, and The McGill CIHR Drug Development Training Program (DDTP).

Poster number: 102

DEVELOPMENT OF NOVEL HCV PROTEIN-PROTEIN INTERACTION INHIBITORS

Martin Baril, Marie-Eve Racine, Jean Duchaine, Anne-Sophie Guenier, Pierre Melançon, Jean-François Lavallée, Stéphane Gingras, Benoit Joliceur, Pierre Lavallée, Daniel Guay Laurent Chatel-Chaix and Daniel Lamarre

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Hepatitis C virus (HCV) infection affects 3% of the population worldwide and is a serious cause of liver disease. For patients infected with genotype 1 HCV, treatment with pegylated interferon (PEG-IFN) and ribavirin is associated with a low success rate and severe side effects over a full year of therapy. In the last 15 years, significant scientific advances have enabled the development of new classes of HCV therapy: direct acting antiviral (DAA) agents, and in 2011 to the approval of first-in-class NS3/4A protease inhibitors for the treatment of hepatitis C, leading to improved viral cure rates and shorter treatment duration. Significant therapeutic advance will be further achieved by a shift to all-oral anti-HCV agents that do not require the administration of ribavirin and IFN-based therapy.

We aimed at the identification of novel classes of DAAs targeting viral protein-protein interactions essential for HCV replication. Since all HCV proteins encompass determinants responsible for their membrane anchoring, we characterized interactions between HCV proteins in live cell assays using Bioluminescence Resonance Energy Transfer (BRET). This technology allowed us to complete a comprehensive HCV protein interaction network analysis of homo- and hetero-dimer associations for all HCV proteins, confirming previously reported interactions as well as identifying novel interactions. We first focused on NS3/4A dimer and NS5A dimer interactions as potential novel druggable HCV targets. Identification of small molecule inhibitors of NS3/4A or NS5A homotypic interactions that synergize with the first generation of NS3 protease inhibitors or emerging NS5A inhibitors may provide significant advantages in a mechanism-based antiviral combination therapy for HCV infection. A chemical biology approach was undertaken for identification of specific modulators of HCV protein-protein interactions. We have established the feasibility of cell-based BRET assays in HTS format and have completed the screening of IRIC-UdeM collection (110,000 compounds) with two selected protein-protein interactions: NS3/4A-NS3/4A and NS5AD1-NS5AD1 homodimers. Following hit-to-lead activities, we demonstrated dose-response micromolar range inhibition of HCV replication in a subgenomic HCV replicon with hit compounds. Medicinal chemistry has been initiated with optimization of a potent antiviral series and has established an emerging structure-activity relationship (SAR). More than 110 analog compounds were synthesized resulting in many derivatives with good antiviral activity (IC_{50} < 1 μM) and with low cytotoxicity (CC_{50} > 20 μM). Comprehensive analysis of the in vitro resistance profile by characterization of HCV resistant replicon variants, through both traditional colonies cloning as well as cell population deep-sequencing, is ongoing with this class of antiviral compounds. No overlapping resistance mutations were observed between our lead compound and drugs presently commercialized or in clinical trials, demonstrating the potential of our approach to identify new therapeutic HCV agents useful in a combination regimen. Our data highlight the identification of compound-induced conformational change of protein dimer interactions with BRET-based assay that lead to a novel class of HCV-specific inhibitors.

Funding source: CIHR-MOP-86485

Poster number: 103

ALTERED THYMIC FUNCTION DURING INTERFERON THERAPY IN HCV-INFECTED PATIENTS

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9. Université Paris Descartes, Faculté de Médecine René Descartes, UMR-S 8104, Paris, F-75014 France.

Background: Interferon alpha (IFNα) therapy, despite good efficacy in curing HCV infection, leads to major side effects, in particular induction of a strong peripheral T-cell lymphocytopenia. However, the mechanisms underlying the establishment of such lymphopenia remain poorly understood. We here analyzed the early consequences of IFNα therapy on both thymic function and peripheral T-cell homeostasis.
Methods: Patients in acute or chronic phase of HCV-infection (n=16) and HIV/HCV co-infected patients (n=10), all naïve to IFNα therapy were enrolled in this study. The evolution of T-cell subsets and T-cell homeostasis were estimated by flow cytometry while thymic function was measured through quantification of T-cell receptor excision circles (TREC) and estimation of intrathymic precursor T-cell proliferation during the first four months following the initiation of IFNα therapy.
Results: Beginning with the first month of therapy, a profound lymphocytopenia was observed for all T-cell subsets, including naïve T-cells and recent thymic emigrants (RTE), associated with inhibition of intrathymic precursor T-cell proliferation. Plasma concentration of interleukin (IL)-7, a homeostatic cytokine, dropped rapidly as lymphocytopenia progressed. This was neither a consequence of higher consumption of the cytokine nor due to its neutralization by its soluble receptor CD127. Decrease in IL-7 plasma concentration under IFNα therapy correlated with the decline in HCV viral load, thymic activity and RTE concentration in blood. These data demonstrate that IFNa-based therapy rapidly impacts on thymopoiesis and, consequently, perturbs T-cell homeostasis.
Conclusions: Such a side effect might be detrimental for the continuation of IFNα therapy and may lead to an increased level of infectious risk, in particular in HIV/HCV co-infected patients. Altogether, this study suggests the therapeutic potential of IL-7 in the maintenance of peripheral T-cell homeostasis in IFNα-treated patients.
Funding sources: This work was supported by Institut Pasteur, the ANRS (Agence Nationale de Recherches sur le SIDA et les hepatitis virales), SIDACTION, the Canadian Institutes for Health Research (CIHR) (MOP-74524), Fonds de la Recherche en Santé du Quebec (FRSQ) (FRSQ-12428) and the FRSQ-AIDS and Infectious Disease Network (SIDA-MI).

Poster number: 104

HCV MODULATION OF CLASS I HUMAN LEUKOCYTE ANTIGENS

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Killer Cell Immunoglobulin-like Receptors (KIR) regulates the function of Natural Killer cells by binding to the ubiquitously expressed class I human leukocyte antigens (HLA). The interaction of KIR with its ligand on normal healthy cells prevents Natural Killer cell activation. Many viruses deliberately interfere with expression of class I HLA molecules to evade the adaptive immune response; however this can result in Natural Killer cell activation and elimination of the virus infected cell. Both class I HLA molecules and KIRs are encoded by highly polymorphic gene families. Clearance of HCV and response to HCV treatment has been linked to an epistatic interaction between particular KIR (KIR2DL3) genes and a subgroup of HLA-C alleles (C1) suggesting that HCV disrupts expression of class I HLA1. However, the effects of HCV infection on class I HLA are not clear. Using the JFH strain of HCV and Huh7.5 cells we have observed downregulation of class I HLA surface expression 7 days post infection in spite of an early rise in messenger RNA. Future studies are planned to elucidate the mechanism of class I HLA loss in culture as well as extend the observations to primary human hepatocytes using the chimeric mouse model. These studies lay a foundation to dissect how the combination of KIR and HLA-C genotype influences the immune response to HCV.

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This work was supported by CIHR Cl6-103134.

Poster number: 105

RESPONSE OF TWO HCV STRAINS TO EXOGENOUS INTERFERON TREATMENT IN CHIMERIC MICE

Ran Chen[1], Yueh-Ming Loo[2], Michael Gale Jr[2], Lorne Tyrrell[1]

*Institutions: 1-Li Ka Shing Institute of Virology, Department of MMI, University of Alberta, Canada; 2-Department of Immunology, University of Washington, Seattle, WA, USA

Background: Hepatitis C virus (HCV) infection represents a major global public health problem. Up to date, it is most likely that interferon (IFN) will remain indispensable in the future of HCV therapy. Factors determining the responsiveness to IFN include both host and viral factors. One advantage of the SCID/beige-uPA chimeric mice (*Nat Med. 2001*) is that we can study the infections of different HCV strains in mice produced with identical donor cells.
Purpose: In donor-matched chimeric mice: 1. to compare the study of IFN responses induced by two clinical isolates of HCV with distinct sensitivities to IFN-based therapy (responder versus null-responder); 2. To study, in parallel, the responses of these 2 HCV strains to exogenous IFN treatment.
Method: The mice, repopulated with single donor human hepatocytes, were divided into 3 groups. Mice in the 1st group were infected with a genotype (gt)-2 HCV strain isolated from a patient who achieved a SVR after pegIFN & ribavirin treatment. In the 2nd group, mice were infected with gt-1 HCV from a patient in whom pegIFN and ribavirin produced less than 1 log drop in viral titers (a null responder). Four weeks after infection, half number of mice in each group received a treatment with IFNa or saline for 2 weeks. A 3rd group of mice were mock infected followed by the treatment of IFN or saline.
Result(s): Both HCV strains produced stable viremia. Surprisingly, unlike the previous results in our SCID-uPA mice which lack the beige trait (*PLoS Pathog 2006*), we did not detect a strong IFN response in mice chronically infected with either HCV strain based on gene-expression analysis. This result was further confirmed in mice produced with hepatocytes from 2 other donors but infected with the same gt-1 HCV strain. Upon treatment with IFN, no significantly declined viremia was observed in the mice infected with gt-1 HCV strain. This is consistent with the non-responsiveness observed in the patient. However, in the mice inoculated with the gt-2 HCV strain, IFN treatment for 2 weeks reduced viral serum levels up to 3-4 logs – similar to the result seen in the patient. Comparative gene expression analysis results indicated that both strains of HCV were able to significantly suppress the level of IFN stimulated genes (ISGs). Interestingly, the responder strain had a similar level of suppression of ISGs as the null-responder strain.
Conclusion(s): In our SCID/beige-uPA chimeric mice produced with the 3 donors in this study, 2 different strains of HCV failed to induce a significant IFN response; Two HCV strains isolated from 2 patients with distinct outcomes to IFN-based treatment were studied. Upon exogenous IFN treatment, the viremia changes in mice infected with the 2 viral strains were consistent with the responses seen in the patients treated with pegIFN & ribavirin; Comparative gene expression analysis results indicated that both strains of HCV were able to suppress ISG expression at comparable levels, indicating that other viral factors play important roles in determining the outcomes of IFN-based therapy since the hepatocytes used in this study were from a single donor.
Funding source: NCRTP-HepC, Canadian Liver Foundation (CLF).

Poster number 106:

USP18 BLUNTS THE ANTI-HCV ACTIVITY OF TYPE I AND III IFN AND INCREASES HCV INFECTIVITY IN VITRO

Limin Chen [1,2,3], Max Xuezhong Ma[1], Bo Qin[2]† , Jing Sun[2], Liang-Tzung Lin[6,7], Christopher D. Richardson[6,7], Nazia Selzner[1,4], Jordan Feld¬[1,5], and Ian D. McGilvray*[1,4]

From the Toronto General Research Institute[1] and the Banting and Best Department of Medical Research[2], Departments of Molecular Genetics [3], Surgery [4,] and Medicine [5], University of Toronto, Toronto, Ontario; the Department of Microbiology and Immunology, Dalhousie University[6], Department of Pediatrics[7], IWK Health Centre, Halifax, Nova Scotia , Canada

Background & Aims: Patients chronically infected by HCV who have upregulation of the ubiquitin-like ISG15 and its protease, USP18, respond poorly to IFN-based treatment. USP18 has both ISG15-dependent and independent effects; having previously shown that its downregulation potentiates the effect of Type I IFN, we investigated whether and how its upregulation alters HCV infection and its susceptibility to both Type I and III IFN.

Methods: Over-expression of wild type (wt) or catalytically inactive mutant (m) USP18 was examined for effects on HCV replication in the absence and presence of IFNα and IFNλ using both the J6/JFH1 infective model and HCV replicon cells (Genotype 1b and 2a). IFN signaling was assessed via Jak/STAT activation (phospho-STAT1 Western blot) and downstream ISG expression (qPCR). Mechanistic roles were sought by quantifying microRNA122 levels and J6/JFH1 infectivity of Huh7.5 cells.

Results: Over-expression of either wtUSP18 or mUSP18 stimulated HCV production and blunted the anti-HCV effect of IFNα and IFNλ in the infective model but not the replicon system. Over-expressed USP18 inhibited neither Jak/STAT signaling nor ISG expression. Consistent with an effect independent of HCV RNA replication, there was no effect on microRNA 122 levels; however, USP18 overexpression markedly increased J6/JFH1 infectivity of Huh7.5 cells.

Conclusions: USP18 stimulates HCV production and blunts the effect of both Type I and III IFN in a manner independent of ISG15 protease activity, Jak/STAT signaling, and HCV RNA replication. Instead, it fosters a cellular environment that promotes HCV infectivity. This study demonstrates how a distinct host ISG response can paradoxically lead to a pro-HCV environment and contribute to treatment failure.

Poster number: 107

ROLE OF TH17 CYTOKINES IN THE PROGRESSION OF LIVER FIBROSIS USING AN IN VITRO MODEL

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Introduction: The majority of individuals infected by hepatitis C virus (HCV) develop persistent infection and many go on to develop liver fibrosis. Hepatic fibrosis is induced mainly by activated hepatic stellate cells (HSCs) that produce extracellular matrix proteins (collagen 1, fibronectin and alpha smooth muscle actin (a-SMA). Factors that induce activation of HSC include cytokines and inflammatory signals caused by viral infections. Several studies suggest that diffrent CD4 + T-cells subpopulations (Tregs, Th1, Th17, Th2) and the cytokines they produce can influence progression of fibrosis. Liver fibrosis is first induced by TGF-ß and can be modulated by anti-inflammatory cytokines such as IL-10 produced by Tregs and IL-22 produced by Th17. However, the role of cytokines and intrahepatic CD4 + T-cells in liver fibrosis is poorly understood.
Hypothesis: The rapid progression of fibrosis observed in some patients is aggrevated by preferential expansion of certain CD4 helper T cell subsets e.g. Tregs and Th17, leading to an imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines, as well as a decrease in lymphocytes producing IL-22 and IL-22 receptor (IL-22R) on hepatocytes.
Methods: We have optimized an in vitro model to examine liver fibrosis using the human stellate cell line LX2 cells. LX2 cells were stimulated with various combinations of cytokines (TGF-ß, IL-17A, IL-22, TNF-a) and relative quantification of gene expression of collagen type I (COL1A1) and a-SMA was performed by real time RT-PCR. The molecules implicated in the firbrogenic process: metalloproteinases 2 (MMP2), inhibitors of MMPs produced by the tissues (TIMPs) and a-SMA were detected by western blot using GAPDH as a standard. Finally, cell surface expression of IL-17RA, TGF-ß-RII and IL-10RB (IL22R, IL10R) on LX2 cell was evaluated by flow cytometry before and after activation by cytokines.
Results: Using our model of activation of HSCs, we demonstrate that the Th17 cytokines IL-17A and IL-22 are not sufficient for initiation of the fibrogenic process within HSCs, as indicated by the lack of induction of collagen type I, α -SMA, MMP2 and TIMP-I . However, in combination with low dose of TGF-ß, which is the first activation signal of HSC, IL-17A and IL-22 have an additive pro-fibrotic phenotype in a dose-dependent manner. This is exemplified by an increase in the induction of the fibrogenic genes COL1A1 and α -SMA and increased protein expression of TIMP-I, α -SMA and MMPs as compared to induction by TGF-ß alone. Moreover, IL-17A, IL-22 and LPS can increase expression level of TGF-ß-RII, IL-10RB and IL-17RA that can lead to increased sensitivity of HSC to activation signals.
Conclusion: Our results suggest that IL17A or IL22 alone have no direct effect on the induction of fibrogenic molecules but may exacerbate the fibrogenic signal induced by TGF-ß via stabilisation of its receptors.
Funding source: This work is supported by grants from the Canadian Institutes for Health Research (***CIHR***).

Poster number 108:

ELUCIDATING NOVEL HEPATITIS C VIRUS/HOST INTERACTIONS USING A MASS SPECTROMETRY APPROACH

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HCV replication and assembly is dependent on virus/host interaction, and is further orchestrated in time and space within the cell. In order to better understand HCV biology and pathogenesis, there is a need to unravel virus/host interaction network. Proteomics studies have been reported using Yeast-two-hybrid, however this system is far from optimal for membrane-bound proteins. Studies undertaken by our group using immunoprecipitation (IP) coupled to mass spectrometry (MS) technique led to the discovery of the NS3-interacting Y-Box binding protein 1 (YB-1) host protein. Since our IP-MS strategy has been proven successful with NS3, we decided to extend our knowledge of virus/host interactions with identification of cellular proteins associated to HCV proteins, and harboring crucial role during its life cycle.

In the first part of the project, FLAG-tagged viral proteins (Core, NS2, NS3/4A, NS4B, NS5A and NS5B) have been expressed in 293T cells, and immunoprecipitated samples have been submitted to MS following ammonium hydroxide basic elution in order to identify host proteins enriched for each viral protein. Bioinformatic analyses were performed using DAVID for Gene ontology (GO) term enrichment and Ingenuity Pathway Analysis (IPA) for host protein interaction networks. Literature mining of described viral-host interactions illustrated the strength of the method, since several characterized interactors were detected in the MS analysis. Selected hits were individually confirmed by Western Blot following FLAG-IP of viral proteins and peptide elution. Using this technique, we confirmed previously described interacting proteins (DDX3 and C1QBP with core, hnRNP A1 and YB-1 with NS3), as well as new HCV/host protein interactions. One example is the immunophilin FKBP51 that has been identified as an NS5B protein interactor. FKBP51 role in HCV life cycle is currently being assessed, since another member of the same family, FKBP8 previously identified as a NS5A interactor, is known to be involved in HCV life cycle.

In the second phase of this project, we will evaluate the role of 150 HCV interactor proteins in HCV replication that were identified in the MS analysis. This will be done by performing a functional RNA interference (RNAi) screen with 5 independent short hairpin RNA (shRNA) sequences. Briefly, Huh7 cells harboring the firefly luciferase sub-genomic replicon are transduced with lentiviral-expressing shRNA targeting gene hits retrieved by MS using the reporter activity readout for HCV RNA replication. Additional screens will be performed using full-length infectious virus to identify MS hits involved in viral assembly/release/infection. Host genes specifically modulating virus life cycle will be further validated for interaction of gene products with HCV proteins by Western blotting and confocal microscopy analysis.

Overall, the studies will lead to the identification of novel virus/host interactions essential in HCV life cycle and provide fundamental knowledge for a better understanding of virus biology and pathogenesis.

Funding source: CIHR-MOP-115058.

Poster number 109:

DIRECT CONTACT WITH HEPATITIS C VIRUS-INFECTED CELLS PROMPTLY INHIBITS NATURAL KILLER CELL FUNCTIONS

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Background: Viral infection requires immune evasion; hepatitis C virus (HCV) succeeds in part by modulating natural killer (NK) cell functions. Previous studies demonstrated NK cell function was decreased by exposure to immobilized HCV particles or overnight incubation with HCV-infected human hepatoma cells.
Purpose: Our current hypothesis is that rapid decrease in NK cell function is due to negative signalling, caused by their direct interaction with infected cells.
Method: We developed a microtitre assay to assess NK cell function in coculture with HCV-infected cells by infecting human hepatoma cells (Huh-7.5) in 96-well plates. Seventy-two hours post-infection, freshly-isolated peripheral blood mononuclear cells (PBMC) and ^{51}Cr-labeled K562 or antibody-coated B cell targets were added and incubated for five hours. Subsequently, cell-based ELISA (CELISA) was performed to assess the degree of virus infection. Effects on NK cytotoxicity were corroborated by flow cytometry (CD107a expression). Briefly, 72hrs post-infection, infected cells were incubated with fresh PBMC and K562 cells for 5 hours in the presence of brefeldin A. PBMC were removed and stained for surface expression of CD3, CD56, and CD107a, fixed, permeabilized then stained for intracellular IFN- γ and analyzed by flow cytometry. To address possible mechanisms of inhibition, we performed chromium release assays with fresh PBMC incubated with α -CD81, or an isotype control, and then compared target cell lysis in the presence or absence of infected Huh-7.5 cells.
Result(s): At high multiplicities of infection and a 60:1 effector to target cell ratio, K562 cell lysis decreased by >20% in the presence of infected Huh-7.5 cells. This effect was corroborated by flow cytometry showing a ~50% decline in NK interferon- γ production and a ~20% decrease in de novo surface expression of the degranulation marker CD107a. Antibody-dependent cell-mediated cytotoxicity against antibody-coated B-cells also declined 10-15%. CELISAs measuring relative HCV protein expression showed that the effect on NK cells correlates with high levels of HCV protein expression. Neither infected cell supernatant nor high titre virus inhibited NK function. This leads us to speculate that the effect on NK cells is due to a direct interaction between NK and HCV-infected cells that delivers a negative signal to the NK cells. We were able to neutralize inhibition of NK function >75% by blocking NK CD81 receptors with α -CD81 then exposing them to infected Huh-7.5 cells. A CELISA with live, unfixed, non-permeabilized HCV-infected Huh-7.5 cells showed some specific binding, suggesting expression of HCV protein(s) on the surface of infected cells.
Conclusion(s): A system was developed to analyze NK function in the presence of HCV-infected cells. CELISA on infected cells after cytotoxic assays showed that inhibition of NK cytotoxicity is proportional to the level of HCV protein expression. Preliminary flow cytometry data show that both NK cytokine production (IFN- γ) and degranulation is reduced by HCV-infected cells. Our working hypothesis is that HCV E2 expressed on the surface of HCV-infected cells interacts with NK CD81 to deliver this negative signal.
Funding source: Research funding provided by CIHR and the Faculty of Medicine, Memorial University. KH is funded by a CIHR Frederick Banting and Charles Best Canada Graduate Scholarship, as well as the NCRTP and the School of Graduate Studies, and RSR is the recipient of a CIHR New Investigator Award.

Poster number 110:

THE ROLE OF HCV NON-STRUCTURAL PROTEIN 5A (NS5A) IN MODULATING VIRAL PROTEIN TRANSLATION

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Background/Purpose: HCV NS5A protein, which is essential for the viral replication, has been implicated in the modulation of viral translation. However, the role NS5A plays in viral translation remains controversial as contradictory studies have been published suggesting that NS5A either stimulates, inhibits or has no effect on viral translation. Possible reasons for these discrepancies include the use of reporter constructs that lack the HCV 3'UTR, where NS5A binds to the poly-U/UC region, as well as use of plasmid encoded reporters that do not accurately reflect the role of the 3'UTR in HCV translation. In this study, we investigated the role of NS5A in the modulation of HCV translation and the function of the 3'UTR poly-U/UC region in this modulation.
Methods: We used monocistronic RNA reporter constructs containing the 5' and 3'UTRs of HCV and an internal Renilla luciferase gene in combination with an NS5A expression plasmid. The HCV 5'UTR contains the internal ribosome entry site (IRES) that drives expression of the internal Renilla luciferase gene, which can be quantified as a measurement of HCV-IRES mediated translation. A **Δ**poly-U/UC construct is used to investigate the role of this region.
Results: NS5A was found to specifically down-regulate viral translation in a dose-dependent manner that requires the presence of the poly-U/UC region of the viral 3'UTR. It was also observed that the three domains of NS5A are capable of modulating viral translation independently. In addition this modulation was found to be independent of the phosphorylation status of the hyperphosphorylation sites found within NS5A. Furthermore this modulation appears to compete with the cellular factor IGF2BP1, which enhances viral translation, for the effect on HCV translation. Also, the viral NS5B protein appears to reduce NS5A down-regulation of viral translation, possibly by binding to it and preventing NS5A from binding to the 3'UTR.
Conclusions: HCV NS5A plays an important role in the modulation of viral translation and the poly-U/UC region of the viral 3'UTR is necessary for this modulation, suggesting that NS5A binding to this region leads to the down-regulation of viral translation.
Funding sources: National Canadian Research Training Program in Hepatitis C, NSERC.

Poster number 111:

MECHANISMS ASSOCIATED WITH GENOTYPE-DEPENDENT, NATURAL RESISTANCE TO NON-NUCLEOSIDE INHIBITORS (NNIS) OF HCV NS5B

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Background: NS5B is the RNA-dependent RNA polymerase (RdRP) responsible for HCV replication. This enzyme is therefore a prime target in current drug discovery and development efforts. However, the different HCV genotypes show variations in susceptibility towards non-nucleoside inhibitors (NNIs), which limits their clinical utility. Nucleoside inhibitors (NIs) bind to the active site of the polymerase and act as chain terminators, whereas non-nucleoside inhibitors act predominantly through allosteric binding sites. At least four distinct binding sites have been identified. These binding sites are not conserved among the various HCV genotypes, which provide a possible mechanism for the observed variations in drug susceptibility.
Purpose: The aim of my research is to test the inhibitory activity of different classes of NNIs with purified NS5B enzymes that represent major genotypes, and to identify the amino acid residues that contribute to a resistant phenotype.
Methods: The inhibitory activity of several NNIs on different genotypes of HCV NS5B polymerase was tested in cell-free assays. We measured the efficiency of primer independent de novo RNA synthesis. *In silico* docking experiments were initially conducted to identify amino acid residues that were present in NNI binding sites. De novo structural models of different genotypes were created using the I-Tasser structure prediction server. By structurally aligning these HCV NS5B models, key residues in genotype 1b were compared with the other genotypes to identify amino acid substitutions that could confer resistance to NNIs. Candidate residues were then tested by directed mutagenesis, followed by biochemical evaluation.
Results: We show that most NNIs tested have potent inhibitory activities towards NS5B genotype 1b, while genotypes 2a, 3a, and 5a show various degrees of resistance. We identified several amino acid residues which were present within 5Å of the NNI binding site of interest. Structural alignments revealed that some of these residues were different in the genotypes resistant to the NNIs. Both single and double mutations were introduced in NS5B resistant to the NNIs. Both single and double mutations were introduced in NS5B 1b, and these mutant enzymes show increased levels of RNA synthesis in the presence of NNIs when compared with the wild type.
Conclusion: The results of this study suggest that several non-1b genotypes display natural resistance to certain classes of NNIs. The identification of the residues that confer resistance may contribute to the rational design of novel NNIs with pan-genotype activity.
Funding Sources: Canadian Institute of Health Research (CIHR), National CIHR Research Training Program in Hepatitis C (NCRTP).

Poster number 112:

ACYLATION OF HCV CORE PROTEIN

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Hepatitis C virus (HCV) core protein is the main structural protein involved in assembly of the viral genome and production of infectious viral particles. Core also plays a significant role in HCV pathogenesis, especially through modification of host lipid metabolism. Core undergoes different post-translational modifications in order to perform its different functions. After maturation of core by sp and spp cleavages, core is modified by the addition of a palmitate group at Cys^{172}, which promotes its association with membranes—a critical step for particle formation. We have shown that the C172S but not C172F mutation affect the association of core to the ER and also abolished ER-LD formation. We also identify a second acylation site, Cys^{184}, in the transmembrane domain of the immature core. Mutation analysis demonstrates that although cleavage of the HCV polyprotein by sp occurs in the absence of Cys^{184} acylation, the latter is necessary for complete maturation of core via spp processing. Consistently, inhibitors of acylation affected processing of core by spp. Stearate moieties seem to be favoured over palmitate to modify the transmembrane Cys^{184}. Identification of post-translational modification of core by the host cell that could be of great interest in the comprehension of HCV infection.

Poster number 113:

MOLECULAR MECHANISMS OF HEPATITIS C VIRUS – ASSOCIATED FATTY LIVER DISEASE

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Hepatitis C virus (HCV) infection is often associated with lipid accumulation within the liver, known as fatty liver disease or steatosis in the clinic. Hepatitis C patients with steatosis are much more likely to progress to more advanced liver diseases, such as cirrhosis and hepatocellular carcinoma. Therefore, prevention of steatosis may represent an important means to retard the progression of HCV-related severe liver diseases. However, the molecular mechanisms of HCV-associated steatosis are not well characterized. Transcriptional factor sterol regulatory element-binding protein-1 (SREBP-1) activates the transcription of lipogenic genes, including fatty acid synthase (FASN). Our recent study has shown that HCV replication can activate SREBP-1 mediated lipogenic pathway. In addition, we have shown that both structural (core) and non-structural (NS2 and NS5A) proteins of HCV can activate this lipogenic pathway through different mechanisms. Our research increases the understanding of the molecular mechanisms of HCV-associated steatosis and will provide rationale for the development of effective therapeutics

Poster number 114:

HCV-SPECIFIC IL-21 PRODUCING T-CELLS ARE INDUCED IN HIV/HCV COINFECTED INDIVIDUALS AND ARE ASSOCIATED WITH HAART THERAPY AND HCV VIRAL CONTROL

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Background: Since the introduction of highly active antiretroviral therapy (HAART), HCV-related end-stage liver disease has become an important cause of morbidity and mortality in those infected with HIV and HCV. Coinfection with HIV enhances HCV RNA loads and liver damage. The impact of HAART on fibrosis progression is presently unclear as studies have found that HAART slows fibrosis progression while others show antiretroviral therapy to promote fibrogenesis (*1-3*). As well, the underlying immune mechanisms driving faster liver disease progression are unknown. We are investigating whether HIV infection can direct the T-cell immune response of coinfected persons such that it is less effective at containing HCV. Previously, the cytokine interleukin (IL)-21, produced mainly by CD4+ T-cells, was found to prevent CD8+ T-cell exhaustion, leading to the resolution of LCMV infection (*4, 5*) and better control of HIV (6). HIV-specific IL-17A producing cells have been described in early HIV infection and may be aberrant HIV-specific T cells that are primed in acute infection as a result of bacterial translocation (7). We examined the presence of IL-21 and IL-17A-producing T-cells in HCV-monoinfected and HIV/HCV-coinfected individuals who were HAART naïve and those receiving HAART.

Methods: Mononuclear cells, isolated from peripheral blood and liver biopsies were obtained from HIV/HCV-coinfected and HCV-monoinfected individuals during acute and chronic infection. *In vitro* assays including intracellular cytokine flow cytometric staining, tetramer staining, and multiplex assays assessed T-cell responses to HCV antigens measured by expression of IL-2, IL-17A, IL-21 and IFN-γ. All immunological findings were related to clinical data including HIV viral load; HAART status; $CD4^+$ T cell count; HCV viral load, age and liver disease markers.

Results: Longitudinal analysis indicates that during acute HIV/HCV coinfection, subjects with HCV control show a transient expansion of IL-21-producing HCV-specific CD4+ T-cells.

Surprisingly, chronic HCV monoinfection was characterized by significantly less IL-21 secretion by HCV-specific CD4+ T-cells when compared to HAART-treated HIV/HCV-coinfected subjects (p=0.0078*)* although this effect was not observed in HAART-naïve subjects (p=0.0931*)*. As well, chronic HCV monoinfection was characterized by significantly less IL-17 secretion by HCV-specific CD4+ T-cells when compared to HAART-treated coinfected subjects (p =0.0420*)* but not in HAART-naïve coinfected subjects (p =0.1331). In an individual with HCV monoinfection we examined *ex vivo* PBMC and intrahepatic lymphocytes isolated from a liver biopsy showing Metavir stage 3 fibrosis. In this individual, we found IFN-γ and IL-17, but no IL-21 production in PBMC in response to HCV antigens and greater IFN-γ and IL-17 production to HCV antigens compared to IL-21 production intrahepatically.

Conclusion: The finding that IL-21 is secreted by HCV-specific T-cells in HIV/HCV-coinfected individuals with apparent viral control may lend support for the development of IL-21-based therapies to enhance the early response to HCV and promote viral clearance during the acute phase of infection. Stronger IL-21- and IL-17 producing T-cell responses in chronically HIV/HCV coinfected individuals may provide new insight into the mechanism of enhanced fibrogenesis observed in these persons. Future studies comparing *ex vivo* PBMC and intrahepatic lymphocyte responses will be carried out to further investigate our findings.

Funding Source: NCRTP-HepC Postdoctoral Award

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Poster number 115:

INVESTIGATING THE MODULATION OF FATTY ACID SYNTHASE (FAS) ACTIVITY BY HCV USING ACTIVITY-BASED PROTEIN PROFILING

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Hepatitis C virus induces lipid biogenesis and accumulation of lipid droplets (LDs) to facilitate its viral life cycle. In situ studies have shown that HCV modulates the expression of proteins involved in fatty acid biosynthesis, including fatty acid synthase (FAS), a multi-domain enzyme that plays a key role in the synthesis of fatty acids and generation of LDs. Activity-based protein profiling (ABPP) represents a unique tool to interrogate the intricate alterations in the FAS activity caused by HCV infection.
Purpose: Investigate the differential activity and expression of FAS during HCV infection
Method: Orlistat and C75, which are two known inhibitors of FAS, have been converted into activity-based probes against FAS and have been successfully employed to target this enzyme in human hepatoma (Huh7) cells.
Result(s): Differential activity in the host proteome has been detected in the presence of the HCV subgenomic replicon and some of its individual structural and non-structural proteins.

Poster number 116:

HEPATOMA CELLS INFECTED WITH HEPATITIS C VIRUS SECRETE VLDL WITH LESS TRIACYLGLYCEROL CONTENT AND HAVE A DEFICIENCY IN A PUTATIVE TRIACYLGLCYEROL LIPASE, ARYLACETAMIDE DEACETYLASE

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Aims: There is compelling evidence that hepatocellular lipid droplets (LDs) and VLDL (very low density lipoproteins) secretory pathway play and essential role in the production of infectious HCV by cell cultures. The hydrophobic core of LDs consists mainly of triacylglycerol (TG) and the majority of lipid that is assembled and secreted with hepatic VLDL is derived from lipolysis and re-esterification of TG stored in hepatocellular LDs. little is known about the lipases that mediate this process. In this study, we examined the impact of HCV infection on VLDL assembly/secretion pathway. We tested the hypothesis that HCV infection reduces the availability of LDs as a VLDL substrate pool.

Methods: Using JFH-1/Huh7.5 cell culture system, the impact of HCV infection on cellular TG levels and secreted TG levels (as VLDL) was determined after generation of a large intracellular TG store (as LDs) by supplementing the culture media with oleic acid. Other cellular factors relevant to hepatic VLDL assembly/secretion pathway were also evaluated including: analysis of apolipoprotein B100 (ApoB: an obligatory structural protein for VLDL) synthesis and secretion (by metabolic labeling and immunoprecipitation and/or ELISA), activity and expression analysis of microsomal triacylglycerol transfer protein (MTP) which primarily transfers TG from cytosolic LDs into the newly synthesized ApoB to form nascent but poorly lipidated VLDL (by MTP activity assay and immunoblot/qRT-PCR analysis) and putative lipases (by activity based probe-labeling and immunoblot/qRT-PCR analysis).

Results: We found reduced secretion of intracellular TG stores in HCV infected cells, which resulted in accumulation of TG in these cells, while synthesis and secretion of ApoB remained unchanged. These results indicate the secretion of VLDL with relatively lower amounts of TG in infected cell culture and were explained by modification of key intracellular regulatory steps involved in the lipidation process of VLDL as both MTP and cellular lipase activities were diminished in lyasates from infected cells. Using activity based probe-labeling assay, we found that labeling of a polypeptide corresponding to arylacetamide deacetylase (AADA) was ablated in infected cells and this correlated with an absence of detectable AADA protein and greater than 95% reduction in cellular AADA transcript abundance. We found that AADA has TG lipase activity in Huh7.5 cells.

Conclusion: These results demonstrate altered regulatory steps of VLDL assembly/secretion and reduced availability of LDs for VLDL assembly/secretion and also show a possible role for AADA in this process.

Poster number 117:

ARFP EXPRESSION IS ASSOCIATED WITH HEPATIC STEATOSIS IN TRANSGENIC ZEBRAFISH LIVER

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Background: The nucleocapsid or core protein is thought to be responsible for some of the major pathogenic effects of HCV, including the development of fibrosis, steatosis, cirrhosis, and hepatocellular carcinoma. An alternate translational open reading frame exists in the core gene that allows the synthesis of another protein termed « F protein » or « alternate reading frame protein » (ARFP), the role of which remains poorly understood. Since the presence of ARFP was not ruled out in most studies of core biological functions, it is possible that the roles attributed to core reflect the activity of ARFP.

Purpose: Our objective is to determine the biological functions of ARFP in hepatocytes and their influence on HCV-associated pathogenesis through the study of transgenic lines of zebrafish (Danio rerio) in which the liver fatty acid binding protein (L-FABP) promoter was used to direct the expression of various forms of ARFP, including $AF11_{opti}$ (frameshift at codon 11, codon-optimized) and $AUG26_{opti}$ (initiation at codon 26[+1], codon-optimized).

Methods and results: Founders (F0) were obtained with both constructs by microinjection of transgenesis vectors bearing *Tol2* transposase recognition sites in one-cell stage embryos, followed by screening for liver-specific AcGFP expression. F1 fishes were obtained by crossing F0 with wild-type, and F2 fishes were obtained by breeding F1 lines with wild-type. Germ line integration and expression of ARFP transgenes was confirmed using PCR and RT-PCR in both transgenic lines. Upon reaching maturity, the phenotype of F2 transgenic zebrafishes was analyzed for morphological, histological and microscopic signs of liver-associated pathology, and compared to the results obtained with non-transgenic fishes. Inspection of liver sections using hematoxylin-eosin staining revealed the presence of lipid accumulation consistent with microvesicular steatosis in both $AF11_{opti}$ and $AUG26_{opti}$ lines, as compared to wild-type specimens. These results were confirmed using Oil Red O staining. The nature of the lipids in question was examined using thin layer chromatography. Expression of various genes associated with fibrosis and hepatocellular carcinoma was analyzed by quantitative RT-PCR on total RNA extracted from the liver. Preliminary results showed that the expression of TIMP-2, MMP-2, and p53, but not that of c-myc, were increased in both $AF11_{opti}$ and $AUG26_{opti}$ lines compared to wild-type, suggestive of the presence of ongoing hepatic fibrosis.

Conclusions: These results suggest that targeted expression of ARFP is associated with the development of steatosis and hepatic fibrosis in zebrafish, an animal model that is commonly used in the study of liver development. In addition, because $AF11_{opti}$ and $AUG26_{opti}$ exhibit distinct 5' coding sequences, these results suggest that putative ARFP determinants associated with steatosis and fibrosis are not located within the N-terminal portion of the protein. This line of investigation should help to clarify the role of ARFP in the pathogenesis of hepatitis C and could lead to the development of novel antiviral strategies.

Funding source: Supported by the PHAC/CIHR Research Initiative on Hepatitis C.

Poster number 118:

INCREASED DENDRITIC CELL FUNCTION DURING ACUTE HCV INFECTION

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Background: Dendritic cells (DCs) are the major antigen-presenting cells (APCs) of the immune system. They capture and process antigens and migrate between sites of infection and lymph nodes to initiate adaptive T cell responses. DCs rapidly differentiate into mature DCs in response to various "danger" signals like toll like receptor (TLR) ligands. This maturation process involves up-regulation of several co-stimulatory molecules to optimize antigen presentation and T cell activation. The role and function of DCs in chronic HCV infection remains highly controversial. Various groups have reported that DCs are defective in chronic HCV in particular in response to TLR ligands, while others have demonstrated that they are functional.
Purpose: We have previously studied the role of NK cells during acute HCV but the role of DCs during the acute phase has not been studied yet. We hypothesize that DCs will be dysfunctional in HCV patients that progress to chronicity.
Method: To examine the function of myeloid DCs (mDC) we have optimized a flow cytometry based method to study the frequency, maturation status, cytokine production and endocytic capacity of mDCs in response to a TLR4 ligand (LPS) and a TLR8 ligand (ssRNA). mDC were studied in a cohort of intravenous drug users exposed to HCV. Three groups were studied: acute HCV with chronic evolution (n = 15), acute resolving HCV (n = 10) and healthy donors (n = 15). Three time points were studied: early acute (2 months after estimated time of infection), late acute (8 months) and follow-up (1-2 years).
Results: We observed a lower frequency of CD86+ and PDL1+ mDCs in chronics compared to healthy donors during the follow-up phase. In addition, both spontaneous resolvers and chronics produced high levels of IL-12 and TNFa in response to stimulation with ssRNA compared to healthy donors. In contrast, chronics exhibited reduced capacity to produce TNFa in response to LPS stimulation with viral persistence.
Conclusion: mDCs from spontaneous resolvers and chronics are functional in response to ssRNA. Spontaneous resolvers are more functional than chronics in response to LPS suggesting gradual impairment in mDC function with progression to chronic HCV. In future directions, we plan to correlate mDC function with serum levels of LPS and the cross-talk between NK cells and DCs.
Funding: CIHR, FRQS and NCRTP-HepC.

Poster number 119:

STRUCTURAL ANALYSIS OF THE INTRACELLULAR HEPATITIS C VIRUS GENOME 3'-UNTRANSLATED REGION

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Introduction: The hepatitis C virus (HCV) genome consists of a 9.6 kb single-strand positive ss(+) sense RNA encoding a single open reading frame (ORF) flanked by two highly-conserved untranslated regions (UTR). The 5'-UTR is approximately 340 nucleotides in length and possesses a series of complex double-strand (ds) RNA stem-loop (SL) structures that form the internal ribosome entry segment (IRES). The IRES initiates cap-independent translation of the viral genome ORF for synthesis of virion and nonstructural (NS) proteins and for initiation of viral replication. The 3'-UTR is a 240 nt tripartite structure comprising a variable region (VR), a poly(U)/C(U)n-repeat and a highly-conserved 98 nt sequence called the X-tail. Lowest free energy models and *in vitro* chemical cleavage data suggest that the X-tail forms a stable dsRNA SL (SL1) and a less ordered 52 nt segment. This latter segment can be modeled as two short hairpin motifs (SL2 and SL3), or as the SL3 motif and an intervening pseudoknot structure. The X-tail and adjacent sequence are essential for *in vitro* synthesis of the HCV (-)RNA strand and for virus infectivity of chimpanzees. The VR segment is dispensable for (-)RNA strand synthesis but appears to enhance RNA replication.
Question: An understanding of the intracellular state of the HCV 3'-UTR is fundamental to our comprehension of HCV replication. From available in vitro data, the 3'-UTR is predicted to form several dsRNA hairpin structures. However, the state of intracellular dsRNA structures as they exist in the 3'-UTR of the complete HCV genome is unknown.
Method: To elucidate this question, the 3'-UTR RNA fragment or the entire HCV genome were crosslinked in situ, and used as a substrate in combination with site-directed RT-PCR to identify regions of dsRNA.
Results: In agreement with prior studies, in situ crosslinking of the 3'-UTR fragment detected dsRNA in the X-tail. *In situ* examination of the genomic HCV 3'-UTR showed that the X-tail structure may be tightly complexed with HCV replication proteins, causing occlusion of crosslinker access to dsRNA. The data also indicate that RNA sequences within the genomic VR-poly(U)/C(U)n-repeat formed dsRNA. Sequences within this VR repeat also appeared to form dsRNA structures with RNA sequences located outside the 3'-UTR region.
Conclusion: These findings provide the first physical evidence for the existence of dsRNA in the 3'-UTR of the intracellular HCV genome and demonstrate the utility of in situ crosslinking and PCR to map regions of dsRNA within large intracellular RNAs.
Funding source: This work was supported by a grant from the Canadian Institutes for Health Research.

Poster number 120:

MIR-122 MEDIATES VIRAL RNA ACCUMULATION IN THE LIFE CYCLES OF BOTH HEPATITIS C VIRUS (HCV) AND GB VIRUS B (GBV-B)

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Hepatitis C virus (HCV) infection is a rapidly increasing global health problem with over 170 million people infected worldwide. A highly abundant, liver specific microRNA, miR-122, interacts with two sites within the 5' noncoding region (NCR) of the HCV genome. This is an unusual microRNA interaction in that it enhances HCV RNA accumulation. This interaction is important for maintaining HCV RNA abundance in both HCV-infected cells and in the liver of infected chimpanzees, suggesting that miR-122 is an attractive antiviral target. In fact, antisense locked nucleic acid inhibitors of miR-122 are currently in phase II clinical trials to assess their efficacy for the treatment of HCV (Santaris Pharma a/s). Our recent work suggests that miR-122 stabilizes the viral RNA, however the mechanism of stabilization as well as how the complex between miR-122 and the viral RNA differs from that with the microRNAs normal cellular targets is still unknown.

We recently found that a closely related virus, GB virus B (GBV-B), first isolated from tamarins injected from serum from a human hepatitis patient, also contains conserved miR-122 sites in its 5' NCR. GBV-B is closely related to HCV phylogenetically, is hepatotropic, and causes an acute, self-limiting infection in tamarins. With strong conservation of predicted miR-122 binding sites, GBV-B represents a potential surrogate model for the role of miR-122 in HCV infection. To investigate the role of miR-122 in the life cycle of GBV-B, we have performed extensive mutational and reverse genetics analyses using a GBV-B replicon model. We find that the GBV-B 5' NCR contains two functional miR-122 sites that mediate viral RNA accumulation. Our results suggest a similar mechanism of miR-122-mediated viral RNA accumulation in the life cycles of HCV and GBV-B, but suggest distinct differences in the oligomeric complexes formed between miR-122 and the viral RNAs. This finding may provide insight into this novel mechanism of microRNA action and may help identify novel targets for therapeutic intervention.

Poster number 121:

ACTIVATION OF MEMORY B CELLS IN CHRONICALLY INFECTED HEPATITIS C PATIENTS WITH AND WITHOUT CRYOGLOBULINEMIA

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Background/purpose: Approximately 170 million people worldwide are chronically infected with the hepatitis C virus (HCV) with thousands dying yearly from this disease. A subset of HCV patients will develop B cell lymphoproliferative diseases such as cryoglobulinemia and non-Hodgkin's lymphoma. Cryoglobulinemia manifests as a vasculitis linked with cryoglobulins (immunoglobulins that precipitate at temperatures lower than 37°C) comprising monoclonal or polyclonal IgM, IgG and HCV virions. Conflicting reports exist as to the state of B cell activation in patients with HCV. Thus, the main purpose of this study was to determine if naïve and/or memory B cells are activated in our cohort of HCV patients and whether the B cell phenotype differs in patients with cryoglobulinemia.

Method: Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll gradient from the blood of 54 chronically infected HCV patients and 50 age/sex matched healthy controls. Patients/controls were thoroughly screened to exclude any person taking anti-inflammatory medications or who had a recent vaccination or significant concurrent illness. Each patient was tested for cryoglobulinemia and 14 new diagnoses were identified. Flow cytometry was used to quantify the expression of 6 activation markers (CD86, CD69, CD40, HLA-DR, CD71 and CD183) on fresh B cells where naïve B cells are CD19+CD27- and memory B cells are CD19+CD27+. Study protocols were approved by the Health Research Ethics Board at the University of Alberta and each donor signed an informed consent.

Results: We found that memory B cells were strongly activated in HCV patients with significant upregulation of CD86, CD69, HLA-DR, CD71 and CD183 compared to healthy controls ($P<0.01$). The levels of most activation markers correlated with liver disease but did not correlate with HCV serum titers, treatment history or a specific genotype. In contrast, naïve B cells were not significantly activated in chronically infected HCV patients. In our study, 37% of HCV patients had detectable cryoglobulins, but we did not find significant changes in B cell numbers and naïve/memory subset percentages. Interestingly, there was significantly higher expression of CD86, CD71 and HLA-DR on memory B cells when cryoglobulin positive patients were compared to HCV patients without cryoglobulinemia ($P<0.05$). In addition, we found a positive correlation between the amount of cryoglobulins (% cryocrit) and memory B cell expression of HLA-DR ($r=0.4523$, $P=0.044$).

CONCLUSIONS: Our findings demonstrate memory B cells are predominantly activated in HCV patients with greater activation seen in patients with cryoglobulinemia. Investigations are ongoing to define the mechanism of activation of B cells and IgM rheumatoid factor production associated with cryoglobulinemia.

Funding sources: Canadian Excellence Research Chair (CERC) in Virology (M. Houghton) and Alberta Innovates-Health Solutions (AIHS) Fellowship (D. Santer).

Poster number 122:

OCCLUDIN, CD81 AND CD5 ARE ESSENTIAL FOR HCV INFECTION OF T CELLS, WHILE CLAUDIN-1 AND SR-B1 ARE DISPENSABLE

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Background: Infection of T cells with wild-type HCV, although occurring at low levels has been well documented. The phenomenon of HCV persistence might be attributed to HCV latency in immune cells including T cells. However, factors involved in HCV entry and replication in T cells are not yet identified. To determine if the previously proposed HCV receptors may play a role in T cell infection, we characterized the expression of CD81, SR-B1, CD5, claudin-1 (CLDN-1), and occludin (OCLN) in HCV-susceptible and nonsusceptible lymphoid cells in comparison to hepatoma-derived cells and primary human hepatocytes (PHH). Moreover, we identified their level of expression before and after exposure to HCV.

Methods: HCV-susceptible Molt4, Jurkat, affinity-purified human T cells, total PBMC and HCV-resistant PM1and CEM T cells were examined for the expression of different HCV candidate receptors in comparison to hepatoma-derived cells (Huh7.5 and HepG2 cells) and PHH as well as 293-HEK cells.

Results: All cells except HepG2 cells express CD81. Although, SR-B1 mRNA was expressed by all cells examined, only HCV-nonsusceptible T cells (PM1 and CEM) as well as hepatoma-derived cells, PHH and 293-HEK cells expressed SR-B1 protein. Interestingly, OCLN was expressed only by HCV-susceptible T cells, however, the level of expression was significantly lower than hepatoma derived cells, PHH and 293-HEK cells. CLDN1 was not expressed by all lymphoid cells tested except the HCV-nonsusceptible PM1 cells. CD5 was only expressed by HCV-susceptible lymphoid cells and no protein was expressed by hepatoma-derived cells, PHH and 293-HEK cells. Blocking of CD81 or CD5 using mAb decreased the susceptibility of T cells to HCV infection. Further, knocking down CD5 and OCLN using shRNA-encoding lentiviral particles were associated with inhibition of HCV infection of T cells. Consequently, infection of T cell lines with plasma-derived HCV downregulated CD81 and OCLN but upregulated CD5, but no change was detected in the level of expression of SR-B1 mRNA.

Conclusion: While no association was found between the expressions of CLDN-1 or SR-B1 and HCV infectivity of T cells, OCLN, CD81 and CD5 were found to be indispensable, suggesting a role of these molecules in HCV lymphotropism. In addition, in vitro infection of T cells with HCV tends to downregulate CD81 and OCLN which may prevent T cells superinfection, but upregulates CD5 which may offer resistance of infected T cells to apoptosis. Our findings reveal specific cellular factors used by HCV to inhabit T cells which may affect the future strategies for HCV treatment.

Poster number 123:

INNOVATIVE SMALL MOLECULE INHIBITORS OF HCV INFECTIVITY

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Although the first two clinical DAA against HCV are a major breakthrough, the goal of IFN-free therapy which does not select for resistance has yet to be accomplished. There is therefore still a need for novel antiviral approaches. We use chemical biology to characterize functions required for viral replication that can be targeted with small molecules. Using this approach, we discovered a family of non-cytotoxic inhibitors of infectivity of HCV and other enveloped viruses. The compounds act on novel virion targets via novel mechanisms. Based on their structural and biophysical requirements for activity, we named them RAFIs (rigid amphipathic fusion inhibitors). The two leading RAFIs, dUY11 and aUY11 inhibited the infectivity of HCV strain JFH1 to Huh7.5 cells, as evaluated by foci forming assays (IC_{50}, 95 or 180 nM, respectively). RAFIs target virions, not cells. We took advantage of their intrinsic fluorescence to analyze their molecular targets. The fluorescent spectra of aUY11 or dUY11 mixed with HCV or other otherwise unrelated enveloped DNA or RNA viruses were remarkably similar to their spectra mixed with protein-free liposomes or in ocatanol (of similar polarity as the interior of lipid bilayers), but remarkably different to those in aqueous buffer. RAFIs therefore localize to the hydrophobic core of the lipid envelope of HCV and other enveloped virions. dUY11 and aUY11 inhibited pH-dependent or independent fusion of the external leaflets of the virion envelopes to cell membranes. They also inhibited the transition of model lipid membranes from lamellar to HII phases, which is equivalent to the formation of the negative curvature required for virion envelopes to fuse to cell membranes. Treatment with RAFIs of cells previously infected with the model DNA or RNA viruses inhibited the infectivity produced by up to a million-fold. Selection of resistance to RAFIs has not yet been accomplished, despite one year of efforts. RAFIs are therefore a novel type of anti-HCV compounds that act on novel targets via novel mechanisms.

We are also characterizing the anti HCV mechanism of a green tea catechin, epigallocatechin gallate (EGCG). EGCG inhibits HCV infectivity, as evaluated by foci forming assays, whereas the structurally related epigallocatechin (EC) does not. Differing from RAFIs, EGCGC inhibited attachment of HCV JFH1, and unrelated enveloped DNA or RNA viruses that also bind to cellular glycosaminoglycans (GAGs), to target cells, as well as their infectivity, whereas EC did not. EGCG did not inhibit the infectivity of poliovirus, which does not bind to GAGs. EGCG, but not EC, has a similar shape and polarity distribution to heparin, which competitively inhibits the primary attachment of virions to cellular GAGs. Our current model is therefore that EGCG is a heparin-like molecule that inhibits attachment of virions to cellular GAGs.

In conclusion, we have discovered and characterized the antiviral mechanisms of two non-cytotoxic small molecules. We are further characterizing and optimizing these compounds and analyzing their potential as antivirals, and we continue using chemical biology to identify novel small inhibitors of HCV infectivity or replication.

Research funded by the Burroughs-Welcome Fund (BWF), CIHR, AI-HS, and NSERC.

Poster number 124:

SMALL MOLECULES MEDIATED PERTURBATION OF HCV-ASSOCIATED HOST PATHWAYS REVEAL POTENTIAL ANTIVIRAL MODULATIONS IN MIRNA EXPRESSION

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Abstract:MicroRNAs (miRNAs) are a class of endogenous small RNAs that play major regulatory roles in virtually all cellular processes. Aberrant expression of these genes is linked to diseased states with hepatitis C virus (HCV) infection being no exception. The modulations in miRNA expression result from both HCV-induced changes in cellular physiology to propagate the viral life cycle and host-induced innate cellular antiviral mechanism. In order to characterize the host anti-viral signature, a comprehensive miRNA microarray analysis was performed using human hepatoma cells treated with small molecules which target pathways associated to HCV pathogenesis. Several differentially expressed miRNA candidates were found in cells treated with either lovastatin, 25-hydroxycholesterol, or benzamide – both small molecules which have previously been shown to inhibit HCV. These perturbations of miRNA profiles may reflect the anti-viral modulation of the host miRNA milieu, and may also elucidate miRNAs playing novel regulatory roles in pathways essential to HCV. Further comparisons between small molecule induced miRNA profiles obtained in the presence and absence of HCV allowed for identification of those miRNAs whose differential expression is sustained in the presence of the virus and, therefore, more likely to play a role in the small molecule's inhibitory effect on the virus. Validation of these differentially expressed candidates is performed by qRT-PCR. The antiviral capacity of these differentially expressed miRNAs as either miRNA mimics or targets for antisense oligonucleotides will be examined on SGR, FGR, and HCV-infected cells.
Funding source: NCRTP-HepC, NSERC, and University of Ottawa.

Poster number 125:

INFECTION OF HUMAN CD4+ AND CD8+ T LYMPHOCYTES WITH MOLECULARLY INTACT HEPATITIS C VIRUS

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Background: Accumulated molecular and clinical evidence indicate that hepatitis C virus (HCV) propagates not only in hepatocytes but also in immune cells. To study HCV-immune cell interactions and potential direct effects of HCV on T cell function, an *in vitro* HCV replication system has been previously established in which mitogen-induced T cell cultures derived from peripheral blood mononuclear cells (PBMC) served as targets for plasma occurring, wild-type HCV.

Purpose: To advance investigations on the interplay between molecularly intact HCV and individual T cell subsets in the absence of pressure exerted by other immune cells, the development of HCV replication system using affinity-purified normal human CD4+ and CD8+ T lymphocytes was the focus of this study.

Method: Plasma from 7 patients chronically infected with HCV genotype 1, 3 or 4, carrying viral loads between 1.5 x 10^3 and 3 x 10^7 vge/ml, were found to be infectious to PBMC-derived T cell cultures. To optimize conditions for infection of virus-naive CD4+ and CD8+ T cells (>97% pure by flow cytometry), HCV inocula most efficiently infecting total T cells were employed. Affinity-purified normal human CD4+ and CD8+ T lymphocytes were pre-stimulated with PHA (5 µg/ml), exposed to HCV and cultured under alternating stimulation with PHA and/or interleukin-2 (IL-2) for 14 days post infection (d.p.i.). HCV RNA positive (genomic) and negative (replicative) strands were detected by strand-specific RT-PCR followed by nucleic acid hybridization (RT-PCR/NAH). Intracellular HCV NS5a and core proteins were identified by confocal microscopy. HCV RNA-reactive particles released to culture supernatants were examined by gradient ultracentrifugation.

Result(s): HCV RNA positive and replicative strands, as well as NS5a and core proteins were detected in both CD4+ and CD8+ T cells after infection with wild-type HCV. HCV RNA-reactive particles displaying distinct sedimentation velocity and buoyant density occurred in inocula and culture supernatants from CD4+ and CD8+ T cells exposed to HCV-positive plasma.

Conclusion(s): Molecularly intact HCV can infect and establish productive replication in normal human CD4+ and CD8+ T cells, as evidenced by detection of HCV RNA replicative strand and intracellular expression of NS5a and core proteins. *De novo* infection with HCV of these two T cell subsets was confirmed by identification of distinct physical properties of HCV RNA-reactive particles in cell culture supernatants and those occurring in infectious inocula. In vitro infection of normal human CD4+ and CD8+ T lymphocytes by molecularly intact HCV should represent a valuable tool to further examine the nature of HCV lymphotopism and how HCV may directly influence the fate and function of these immune cells.

Funding source: National CHIR Research Training Program in Hepatitis C.

Poster number 126:

PROPAGATION OF HEPATITIS C VIRUS IN HUMAN HEPATOMA HEP3B CELLS

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Background: A key challenge in studying Hepatitis C Virus (HCV) is the limited availability of model systems. Huh7 is the only cell line known to be naturally permissive for the complete replication cycle of HCV, and consequently nearly all cell culture research is carried out in Huh7 cells and their derivatives. This places researchers at a disadvantage by limiting our ability to identify new infectious viral clones. It also reduces our ability to study virus-host interactions as we are restricted to HCV interactions with cells from a single genetic background; thus, alternate models for HCV are needed. Part of the natural permissiveness of Huh7-derived cells for HCV is due to expression of microRNA-122 (miR-122), a liver-specific and liver-abundant microRNA that plays a role in supporting the HCV life cycle. Interestingly, most other liver cell lines have lost expression of this microRNA. We hypothesized that lack of miR-122 expression limits the ability of non-permissive cell lines to support HCV.
Result(s): We examined commonly-used human liver cell lines for miR-122 content using qRT-PCR, and found that only Huh7-derived cells (Huh7.5) express detectable levels of miR-122. Thus, we hypothesized that supplementing the non-permissive cell lines with miR-122 would render them to permissive to HCV. By electroporating Hep3B human hepatoma cells with miR-122 and either sub-genomic or full-length HCV RNA, we determined that replication as measured by reporter gene expression, northern blot, and viral titer (with full-length RNA) is similar to levels achieved with parallel experiments in Huh7.5 cells. When Hep3Bs are electroporated with miR-122, and then infected with cell culture-derived viral particles, infection efficiency is much lower than with Huh7.5 cells. Nonetheless, miR-122-supplemented Hep3B cells infected with HCV do subsequently produce virus particles that can infect both Hep3B and Huh 7.5 cells.
Conclusions: We have identified miR-122 as a factor in determining the permissiveness of other human hepatoma cell lines to HCV. We have also determined that Hep3B cells can be rendered permissive to the complete cycle of Hepatitis C Virus infection – from infection, through RNA replication, to production of infectious particles – by supplementation with miR-122. Although the levels of RNA replication and infectious particle production are similar in Hep3B cells and in Huh7.5 cells, attachment and entry of the virus into Hep3B cells appears to be less efficient and begs further exploration. Additionally, Huh7-derived viral particles have a different lipid composition than patient-derived virus particles and we wish to determine the protein and lipid composition of HCV particles derived from Hep3B cells. As these cells have a different genetic background than Huh7 cells, Hep3B cells may prove useful in verifying and analyzing virus-host interactions identified in Huh7-derived cell lines.
Funding sources: SHRF, NSERC, and NCRTP-HepC.

Poster number 127:

HUMAN DICER AND TRBP ARE REQUIRED FOR MICRORNA-122 REGULATION OF HCV TRANSLATION AND RNA ABUNDANCE

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Abstract: The liver specific Micro RNA, MicroRNA-122 (miR-122) promotes Hepatitis C Virus (HCV) RNA accumulation and stimulates HCV translation through physical interaction with tandem binding sites located in the 5' un-translated region (5'UTR) of the HCV genome. To further elucidate the role of RNA induced Silencing complex (RISC) proteins in activity of miR-122; we have investigated the requirement of the RISC loading complex (RLC) proteins, Dicer and TRBP, in HCV replication and in the activity of miR-122, by using siRNA depletion. Dicer depletion reduced HCV RNA abundance in HCV infected cells, and abolished HCV RNA accumulation induced by hairpin pre-miR-122 RNA, but did not affect HCV RNA accumulation mediated by synthetic mature duplex miR-122. In addition, mature, but not hairpin miR-122 stimulated HCV translation in Dicer knockout MEFs, confirming a role in miR-122 processing in both miR-122 mediated HCV RNA accumulation and translation stimulation. TRBP was required for efficient HCV RNA accumulation by both miR-122 hairpin and duplex RNA and is consistent with its proposed role in RISC loading. Thus, for the process of miR-122 augmentation of HCV accumulation and translation, Dicer and TRBP appear to be required for their canonical pre-miRNA processing and RISC loading functions.

Funding Source: NSER.

Epidemiology and Public Health

Poster number 300:

MORTALITY IN A LARGE COMMUNITY-BASED COHORT OF INNER CITY RESIDENTS IN VANCOUVER, CANADA

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Background: Inner city populations with endemic injection drug use are often characterized by high rates of mortality. Major causes of death include consequences of drug use such as drug overdose, suicide, trauma, and HIV. The impact of hepatitis C virus (HCV) infection on mortality in inner city populations is not well-described. The aim of this study was to evaluate causes of mortality among inner city residents with a high prevalence of HCV infection.

Methods: The CHASE study is a community-based cohort study of inner city residents recruited in the Downtown Eastside of Vancouver, Canada from January 2003 to June 2004. At the time of recruitment, participants completed a short, interviewer-administered questionnaire that gathered information on demographics, health service utilization, and recent drug use (previous six months). Retrospective and prospective linkages were established with provincial virology and mortality databases. ICD-10 codes were used to determine causes of death including HIV-related, liver-related, and drug-related mortality.

Results: Among 2,913 participants enrolled (mean age 43; 29% female), 80% (n=2,337) and 38% (n=1,114) had recently used illicit and injecting drugs. Among those with available HCV (n=2,405) and HIV (n=2,270) testing, HCV and HIV prevalence were 64% (n=1,533) and 24% (n=534), respectively. Death occurred in 13% of participants (n=387), over a median follow-up of 6.5 years (17,631 person-years). Causes of death were HIV-related in 21%, drug-related in 14%, liver-related in 6%, and related to other causes in 59%. The all-cause mortality rate was 219 per 10,000 person-years [95% confidence interval (95% CI): 199, 242] overall and 262 per 10,000 person-years (95% CI: 231, 297) in those with HCV infection. Overall, HIV-, drug- and liver-related mortality rates were 45, 32 and 12 per 10,000 person-years, respectively. Among HCV-infected participants, HIV-, drug- and liver-related mortality rates were 72, 50, and 20 deaths per 10,000 person-years, respectively. Liver-related rates of mortality among HCV-infected participants <35, 36-40, 41-45, 46-50 and >50 years of age were 5 (95% CI 1, 29), 11 (95% CI 3, 39), 13 (95% CI 5, 39), 22 (95% CI 9, 56), and 59 (95% CI 30, 116) per 10,000 person-years, respectively. This nearly 12-fold increase in liver-related mortality between the <35 year and >50 year groups contrasts with the overall mortality rate in the same groups, which increased approximately 3-fold from 151 (95% CI 105, 216) to 448 (95% CI 349, 576) deaths per 10,000 person-years.

Conclusions: In this analysis of a large cohort of inner city residents in Vancouver, mortality rates attributed to HIV, illicit drugs and liver-related causes among inner city residents were high, particularly among those with HCV infection. In those with HCV, liver-related mortality was particularly high in those greater than 45 years of age. Strategies are needed to address HCV-related liver disease among older inner city residents with HCV as those infected in the late 1990s continue to age and contribute to the growing burden of liver disease.

Poster number 301:

TREATMENT OF HEPATITIS C IN ACTIVE INTRAVENOUS DRUG USERS (IDUS): VERY HIGH ADHERENCE IN TACTIC PROJECT, A LOW THRESHOLD COMMUNITY MODEL OF CARE IN QUEBEC CITY

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In Canada, more than 70% of hepatitis C new infections involve IDUs. Despite they represent the largest reservoir of infection, less than 2% of active IDUs are treated in Quebec City. Major concerns in treating active IDUs are unstable lifestyle, psychiatric co-morbidities, adherence and risk of re-infection.

Goals of TACTIC project are to provide hepatitis C treatment for active IDUs in a proper context and evaluate adherence. We also document impact of treatment on drug use and incidence of re-infection.
Health services are provided in community setting which regroups a needle exchange program and homeless shelter. TACTIC model of care is based on harm reduction approach and low threshold access to care in proximity where IDUs live. Health care and treatment are delivered by a small multidisciplinary team: 1 family physician with experience in hepatitis C treatment, 2 nurses and 1 outreach community worker. All of them are familiar with IDUs. Engagement of patient is essential.

Preliminary results

38 patients completed treatment with peginterferon and ribavirine (or where stopped by the physician for medical reasons) between June 2009 and December 2011. No drop outs.

Baseline characteristics

Male	23
Female	15
Mean age	37 (23-56)
Stable partner	20%
Unemployment	95%
Prior incarceration	80%
Psychiatric comorbidities	71%
Opiate substitution	24%

All of them injected drugs in the last 6 months prior treatment and half of them injected in the previous week.

Genotypes 1-4	60%
Genotypes 2-3	40%
HIV co-infection	11%
RNA negative end of treatment (n=34)	80%
Sustained virological response (n=25)	68%

Adherence is evaluated by « self-report » questionnaire every 4 weeks and direct questioning by nurses and physician every visit. We collect datas for presences to appointments. Questionnaire was adapted from Virahep-C questionnaire, ACTG and Brief medication questionnaire.
Adherence was defined by: % weeks completed vs prescribed, % doses of ribavirine taken vs prescribed and % doses of peginterferon taken vs prescribed.

We have results for 34 patients (results for 4 patients are missing: 1 died of car accident at beginning of treatment, 2 were hospitalised early and 1 refused to answer).

Adherence (%weeks/%riba/%peg)	N	**%**
>100/95/100%	32	94%
98/89/98%	1	3%
79/-/79% (acute hep C, no Ribavirin)	1	3%

Conclusions: Despite « self report » questionnaire can overestimate adherence, those results show that active IDUs can be successfully treated for hepatitis C with a very adherence rate in a proper context: community based setting, low threshold access to care, harm reduction approach and multidisciplinary team.

*The project is ongoing and is now under evaluation of public health agency. Protease inhibitors will be used as soon as reimbursement will be available.

*No direct funding for study but community based organisms received grants (Point de Repères and L'Auberivière) for the outreach worker from Roche Canada.

Poster number 302:

PERSISTENTLY SERONEGATIVE HCV INFECTION FOLLOWING MOTHER-TO-CHILD TRANSMISSION

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Background: HCV infection typically leads to the development of antibody responses within weeks following primary infection. Here we describe the atypical case of a child who acquired HCV infection by mother-to-child transmission and who remained persistently HCV-seronegative despite the presence of elevated levels of plasma HCV RNA.

Methods and Results: Born 03/1999 to an HCV-1a-infected mother who was not a carrier of HIV-1, this child was diagnosed with HCV in 2000 on the basis of positive PCR assays. Viral load was 997,000 UI/ml, 576,000 UI/ml and 76,000 UI/ml at age 5.25, 9.33, and 9.67 years, respectively. Testing for anti-HCV antibodies using ELISA and RIBA was repeatedly negative (11 times) despite the presence of antibody responses against routine childhood immunizations (HAV; HBV; tetanus; mumps; rubella) and no apparent abnormalities in IgG profiles. Measurement of HCV-specific cell-mediated immune responses using IFN-□ ELISpot and a panel of overlapping peptides representing core, NS3, and NS5B revealed an absence of significant HCV peptide-driven IFN-□ production (i.e. =10 SFU per 10^6 PBMC). Liver histology showed mild fibrosis. High ALT and AST levels justified introduction of treatment with PegIFN-□+ ribavirin at age 9.5 years. Treatment was stopped at age 10.0 years because of absence of sustained virological response. Treatment did not lead to HCV seroconversion. No treatment-related symptoms or side effects were reported. Examination of the HCV variant spectrum based on HVR1 was performed by cloning and sequencing at 5 time points over a 10 year period (17-48 E2 recombinants analyzed per time point). Quasispecies profiling based on amino-acid sequences revealed little time-dependent evolution and was characterized by the presence of a single predominant E2 variant that represented =82.4% of the total number of clones analyzed in the study subject. This variant persisted through time and remained largely predominant following PegIFN-□ + ribavirin treatment. Interestingly, this variant also comprised >80% of sequences identified in the mother and was transmitted vertically along with another minor variant.

Conclusions: These results are consistent with absence of selective pressure exerted on HCV E2 sequences, compatible with an absence of humoral and cell-mediated HCV-specific immune responses in this particular HCV-infected subject. Possible mechanisms leading to this exceptionally uncommon situation could include neonatal tolerance potentially associated with *in utero* transmission.

Funding source: Supported by the PHAC/CIHR Research Initiative on Hepatitis C.

Poster number 303:

ACCEPTABILITY AND WILLINGNESS TO PARTICIPATE IN FUTURE HEPATITIS C VIRUS VACCINE TRIALS AMONG YOUNG PEOPLE WHO INJECT DRUGS IN SYDNEY

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Background: Little is known about the attitudes of young people who inject drugs (PWID) towards immunisation, barriers to uptake and completion of vaccine schedules, and willingness to participate (WTP) in future vaccine trials. This paper reports on acceptability and WTP in candidate hepatitis C virus (HCV) vaccine trials among young PWID in Sydney.
Methods: The Hepatitis C Incidence & Transmission Study – community (HITS-c) is an ongoing prospective community-based cohort of anti-HIV and anti-HCV negative PWID. Attitudes towards immunisation, barriers and facilitators of vaccine trial participation and WTP were assessed at baseline (n=102). Following baseline assessment, participants receive a brief intervention, covering principles of immunisation and key clinical trial concepts. Acceptability, knowledge and WTP were again assessed at six months.
Results: The median age of participants was 27 (range 16-51 years) and the majority (73%) were male. High proportions (98%) reported they would seek immunisation against HCV if a safe and efficacious vaccine was available at no cost. WTP in a preventative HCV vaccine trial was 80%.
In univariate analysis, those who mainly injected methamphetamine were significantly more likely to indicate WTP in future trials compared to those who mainly injected heroin. WTP was also associated with ethnicity, current opioid substitution therapy and serological evidence of hepatitis B vaccine-induced immunity. Gender, age, education, injecting frequency and receptive syringe sharing were not significantly associated with WTP.
Conclusions: Results suggest that drug of choice may be an important determinant of WTP in future trials of candidate vaccines among young PWID. The literature to date has mainly focused on older PWID and primary opioid injectors, however our results suggest that main drug injected may be an important determinant of WTP in future trials of vaccines among young PWID. Potential challenges in ensuring interest and participation in field trials of candidate vaccines will be discussed.

Clinical Sciences

Poster number 400:

PEGYLATED INTERFERON ALFA, NITAZOXANIDE, TELAPRAVIR, RIBAVERIN, IN GENOTYPE 1 UNDERGOING PRIOR EXPERIENCED CHRONIC HEPATITIS C PATIENTS -- A RANDOMIZED PLACEBO CONTROL CLINICAL PILOT TRIAL (I N T R I G U E C) INTERIM

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Objectives: Chronic Hepatitis C is a global challenge with End stage liver disease and rising Hepatocellular Carcinoma. Peg Interferon Alfa and Ribavirin was the backbone of therapy. Recently introduced Directly Acting Antivirals (DAAs) -protease inhibitor has a promising role in escalating Sustained Viral Response (SVR) in Response guided therapy in non-responders, partial and relapses. This study utilized Nitazoxanide (NTZ) & Telapravir, with SOC for 24 weeks in treatment Experienced Patients. Methods: Fifty (n=50) patients were divided into Group A (n=12) NTZ 500mg three times for 12 weeks, Group B (n=12) NTZ 500 mg twice daily for 24 weeks Group C (n= 26) control. All received Peg Interferon Alfa 2a 180 mcg SQ QOW with fixed dose of Ribavirin 1200 Mg daily for 24 weeks with Telapravir 750 Mg three times daily for 12 weeks. Viral load was obtained at day 0, 7th day, 14th day, 4 weeks, 12th week and 24 weeks. Viral kinetics was analyzed.
In Group A: 5/12(42%) Non Responder, 6/12(50%) partial responder, 2/12(16%) relapsers. In Group B: 5/12(42%) Non responders, 6/12 (50%) partial responder, 1/12 relapsers(8%).In Group C: 10/26(38%) non-responder, 10/26 (38%) partial responder, 4/26(15%) relapsers, 2/26(8%) unknown.
Exclusion: Decompensated Cirrhotic,HCC,poor DM,Hemolytic Anemia, Severe Coronary artery disease, major depression, renal failure, Prior severe skin rash, active drug and alcohol abuse.

Table 1

	Group A	Group B	Group C
Non-Responders	5/12(42%)	5/12 (42%)	10/26(38%)
Partial Responders	6/12(50%)	6/12 (50%)	10/26(38%)
Relapsers	2/12(16%)	1/12 (8%)	2/26(8%)
Unknown	NA	NA	2/26(8%)

Table 2: Results

Results	Group A	Group B	Group C
Undetectable	9/12(75%)	10/12(83%)	16/26(62%)
NR	1/12 (8%)	2/12 (16%)	4/26 (15%)
PR	1/12 (8%)	12/12(100%)	3/26 (11%)
AVR	11/12(92%)	12/12(100%)	20/26(77%)
VRVR	11/12(92%)	10/12(83%)	22/26(84%)
RVR	9/12(75%)	10/12(83%)	18/26(70%)

EVR	9/12(75%)	10/12 (83%)	16/26(62%)
ETVR	9/12(75%)	10/12(83%)	16/26(62%)

NR: Non responders and PR : Partial Responders

Side Effects: Anemia 28/50(56%), Neutropenia 14/50(28%) , Thrombocytopenia 8/50(16%), Fatigue 34/50(68%),Depression 10/50(20%), Mild skin rash 22/50(44%), Severe skin rash 1/50(2%).
Use of Growth factors:
Epogen 12/50(24%) Neupogen 8/50(16%) Elthrombopag 5/50(10%

Conclusion: This quadruple truncated regimen has excelled the RVR,ETVR over SOC with DAAs over 13%, without any difference between 24 weeks of NTZ over 12. Needs a larger trial for validation.

Poster number 401:

RETROSPECTIVE ANALYSIS OF PAN ASIAN OUTCOME OF RESPONSE TO THERAPY FOR HEPATITIS C - A MULTICENTER STUDY (REPORT-C STUDY) – INTERIM RESULTS

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Background: Pegylated Interferon Alfa (PEG IFN-a) and Ribavirin is the standard of care in treatment of Hepatitis C (HCV) with overall Sustained Viral Response (SVR) of approximately 50%. SVR varies based on demographics and ethnicity. African Americans and non-white Latinos have lower SVR (27- 33%). There is limited data in Asians.
PURPOSE: This multi-center study retrospectively analyses HCV therapeutic response in a broad Asian population from 1998-2008.
Methods: 161 patients treated for HCV between 1998-2008 were included in the study {New York(USA)-97(60.24%); Hong Kong-41(25.46%); Cleveland (USA)-23(14.28%)}.Population characteristics: Mean age- 50.4 years; Male- 102(63.35%); Female –59 (36.64%). Data was collected electronically and processed in New York City per protocol. Both pegylated and standard interferons with Ribavirin were used for 48weeks for genotype 1, 4 and 6 and 24weeks for genotypes 2 and 3. Modes of transmission, demographics by genotype and interferon subtypes were tabulated.

Table-1 (n, %of N)

Chinese		**Indian Subcontinent**		**Far East**		**Central Asia**		**Middle East**	
Chinese	41 **(25.5%)**	Bangladesh	5	Malaysia	2	Afghanistan	2	Palestine	1
		India	18	Phillipines	6	Uzbekisthan	16	Jordanian	1
Asian Unknown	1	Sri Lanka	2	Korea	2	Tajikistan	9	Egypt	3
		Pakistan	13	Taiwan	3	Kazakhstan	3	Kuwait	1
		Nepal	1	Indonesia	2	Ukraine	1	Saudi	1
		Tibet	2	Vietnam	6	Unknown	1	Turkey	1
		Burma	1	Cambodia	4	**Total**	**32(19.9%)**	Unknown	1
		Total	**42(26%)**	Thailand	6			**Total**	**9(5.6%)**
				Singapore	2				
				Japanese	3				
				Total	**36(22.4%)**				

Results: Out of 161 total patients (N=161): Mean BMI-27.125 (unknown-24 patients); Mean ALT – Chinese -154 IU/L, USA – 61 IU/L; Mean Ribavirin dose- 900mg, unknown- 26; SVR was obtained in 92 patients(57%);{Chinese population from Hong Kong- 53.6%; from New York City: 58.8%; from Cleveland(USA):10/23(43%)}.
CONCLUSION: Overall, pan Asian outcome of treatment of HCV as measured by SVR was better than global SVR in African-American and non white latino populations.

Funding source: Not applicable as this is a retrospective study.

Table-2, Modes of transmission

Mode	**n**
Intravenous Drug Use(IVDU)	60(37.3%)
Sexual Male-to-male sex	15(9.3%) 7(5%)
Blood Transfusion	48(29.8%)
Medical Procedures (including surgery)	17(10.56%)
Unknown	21(13%)

Table-3: Demographics by genotype

Genotype	**N (% of N)**	**Demographics**
G1	78(48.44%)	Indian subcontinent-18,Far east-23,Central Asia-20,Middle East-3, Asian Unknown-1
G2	37(23%)	Indian subcontinent 19,Far East 12,Central Asia 6
G3	30(21.7%)	Indian subcontinent 16, Far east 10, Central Asia 4
G4	6(3.7%)	Indian subcontinent 3,Egypt-3
G6	19(11.8%)	Far east- 19

Table-4: Interferon subtypes

Interferon Type	**N (% of N)**
Standard IFN	53(32.9%),
PEG IFN	98(60.8%)
Consensus IFN	1(0.6%)
Unknown	9(5.6%)

Poster number 402:

EFFECTS OF LIDOCAINE 3% GEL DELIVERED RECTALLY IN ANO RECTAL DYSFUNCTION (ARD) INDUCED BY TELAPRAVIR THERAPY IN CHRONIC HEPATITIS C (CHC -C) A RANDOMIZED PLACEBO CONTROL STUDY

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Objective: Telapravir is a Potent Protease Inhibitor, which causes anorectal dysfunction (ARD) comprising Proctalgia, Rectal Ulcers, Hemorrhoids and rectal bleeding. Conventional therapy is suboptimal causing treatment Failure. This study evaluates 3% Topical Lidocaine gel rectal delivery to abate the drug related ARD to avoid treatment failure.
Methods: 52 Patients (mean age 51) were recruited undergoing therapy with Telapravir, Peg Interferon and Ribaviran for CHC-C. 45/52 (86%), with Rectalgia, 8/52 (15%) rectal ulcers, Hemorrhoids 19/52(36%) with bleeding 6/19(31%) without Bleeding 13/19(68%). Group A (n=17) placebo Group B (n=17) hydrocortisone 2.5% Cream and Group C (n=18) Lidocaine 3% Gel foam per rectally twice daily. All underwent Pre and post Proctoscopic evaluation and Ano-rectal manometry.

Table 1

	Group A	Group B	Group C
Rectalgia	3/17 (17%)	8/17 (47%)	17/18(94%)
Rectal Ulcers	0/2 (0%)	1/3 (33%)	2/3 (66%)
Hemorrhoids resolved w/o bleed	1/6 (16%)	2/6 (33%)	5/7 (71%)
Proctocopic examination showing normalization of Mucosa post therapy	4/17 (23%)	7/17 (41%)	17/18 (94%)

Results: Rectalgia resolved in 3/17 (17%), 8/17(47%) & 7/18(94%) for Group A, B & C respectively. Rectal ulcers healed in 0/2 (0%), 1/3 (33%) & 2/3 (66%) for all the above groups. Hemorrhoids resolved in 1/6 (16%), 2/6 (33%) & 5/7 (71%) in all groups. Pre/Post Proctoscopy revealed normal mucosal integrity 4/17(23%), 7/17(41%) and 17/18(94%) above groups. Results of Pre/Post Rx mean scores for pain, Itching and Burning shown on (Table 3). AR Manometry results showed Pre/Post treatment high sphincter tone >4 mm in Group A 2/ 15(8%) and no differences in pre and post treatment, Group B 7/15(41%), 4/15(22%) and Group C 5/15(20%), 2/15(10%) respectively (Table 4).
Side events; Numbness, 4/17(23%) in lidocaine.
Conclusion: Rectally delivered Lidocaine 3 % gel is efficacious, tolerable compared to the SOC and placebo for ARD causing treatment failure, retention & SVR. Larger trial needs to validate this finding.

Poster number 403:

RAPID AND EARLY VIROLOGICAL RESPONSES DURING TREATMENT FOR RECENT HEPATITIS C VIRUS INFECTION: POTENTIAL BENEFIT FOR THE USE OF RIBAVIRIN IN HCV/HIV CO-INFECTION

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Background: The role of ribavirin in improving responses to pegylated interferon (PEG-IFN) is established in chronic hepatitis C virus (HCV) infection, but less so in recent (acute/early chronic) infection, particularly those with HCV/HIV co-infection.
Purpose: This study evaluated early virological decline during recent HCV therapy in HIV negative individuals receiving PEG-IFN monotherapy and HIV positive individuals receiving PEG-IFN/ribavirin.
Methods: The Australian Trial in Acute Hepatitis C (ATAHC) was a non-randomised prospective study of patients with recent HCV infection. All participants received PEG-IFN alfa-2a for 24 weeks; HCV/HIV participants also received ribavirin. Early HCV RNA decline was assessed among adherent participants (>80% PEG-IFN, >80% treatment). Logistic regression identified predictors of RVR (<10 IU/mL) and SVR.
Results: Of 109 treated, 82% were adherent (HCV, n=57; HCV/HIV, n=32). Overall, RVR was 51% (HCV: 55% vs. HCV/HIV: 43%, P=0.323). Factors independently associated with RVR included duration of infection <26 weeks, HCV RNA <5.6 $\log_{10}$ IU/mL at baseline and HCV genotype 2/3 infection. Between baseline and week 12, mean decline in HCV RNA was greater in HCV/HIV participants (PEG-IFN/ribavirin) compared to HCV participants (PEG-IFN) (4.19 vs. 3.32 log10 IU/mL, P=0.029). Greater HCV RNA decline was observed in those treated with RBV, particularly amongst those with an estimated duration of infection >26 weeks and those with unfavourable IL28B genotypes. Adherent HIV negative and positive participants had similar EVR (76 vs. 90%,P=0.102) and SVR (63% vs. 75%,P=0.253), respectively. RVR was highly predictive of SVR (AOR 4.09; 1.49, 11.25).
Conclusion: The results of this study suggest a potential benefit for PEG-IFN and ribavirin combination therapy in maximizing virological responses in HCV/HIV participants with recent HCV, particularly those with a longer duration of HCV infection and unfavorable IL28B genotypes.

Poster number 404:

TMC435 IN COMBINATION WITH PEGINTERFERON AND RIBAVIRIN FOR TREATMENT OF HCV GENOTYPE 1 INFECTION: WEEK 24 INTERIM ANALYSES OF PHASE IIB PILLAR AND ASPIRE TRIALS

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Background and Purpose: TMC435 is an investigational, potent, once-daily, oral inhibitor of the hepatitis C virus (HCV) NS3/4A protease currently in Phase III clinical development for the treatment of patients infected with HCV, in combination with pegylated interferon a-2a (PegIFNa-2a) and ribavirin (RBV). PILLAR (TMC435-C205; NCT00882908) and ASPIRE (TMC435-C206; NCT00980330) are Phase IIb, international, randomized, double-blind studies performed to assess the efficacy and safety of TMC435 administered with PegIFNa-2a/RBV in different populations of patients infected with HCV genotype 1.
Method: PILLAR: Patients were HCV treatment-naïve. ASPIRE: Patients were HCV treatment-experienced, although naïve to direct acting antivirals (DAA). In PILLAR, TMC435 was administered for 12 or 24 weeks with PegIFNa-2a/RBV. The study design also included response-guided treatment in TMC435 patients. TMC435 patients were able to end treatment at Week 24, if they achieved HCV RNA 25 IU/mL detectable or undetectable at Week 4, and HCV RNA 25 IU/mL undetectable at Weeks 12, 16, and 20. All other patients continued PegIFNa-2a/RBV for up to 48 weeks. In ASPIRE, TMC435 was administered for 12, 24, or 48 weeks. The study had fixed total treatment duration of 48 weeks for all arms. We report the results of planned Week 24 interim analyses, including the proportion of patients with undetectable HCV RNA (25 IU/mL undetectable) at Weeks 4, 12, and 24.
Results: PILLAR; In the TMC435 arms, 68–79% of patients achieved a rapid virologic response compared with 5% in the control arm. At Week 12, 91–97% of those treated with regimens containing TMC435 had HCV RNA 25 IU/mL undetectable, compared with 58% in the control arm. At Week 24, 94–97% of those treated with regimens containing TMC435 had HCV RNA 25 IU/mL undetectable, compared with 82% in the control arm. In the TMC435 arms, 79–86% of subjects were eligible to stop treatment at Week 24 based on response guided therapy. Overall, 5% of patients in the TMC435 arm and 13% of patients in the control group met a virologic stopping rule at Week 12 or 24 due to lack of virologic response.
ASPIRE; At Weeks 4, 12, and 24, significantly higher virologic response rates were observed following treatment with TMC435 plus PegIFNa-2a/RBV, compared with placebo plus PegIFNa-2a/RBV. In null and partial responders, higher virologic response rates were observed in TMC435 dose arms. Overall, 6% of patients in the TMC435 arm and 49% of patients in the control group met a virologic stopping rule at Week 4, 12, or 24 due to lack of virologic response. Highest rates of viral breakthrough and virologic failure were observed in null responders, followed by partial responders, with the lowest in relapsers.
Conclusions: TMC435 administered once-daily with PegIFNa-2a/RBV has potent antiviral activity, rapidly achieving undetectable HCV RNA in the majority of patients. The majority of TMC435 treatment-naïve patients in PILLAR were able to shorten total treatment by 24 weeks compared with PegIFNa-2a/RBV alone. Given in combination with PegIFNa-2a/RBV, the safety and tolerability profile of TMC435 was generally similar to that of the placebo control.

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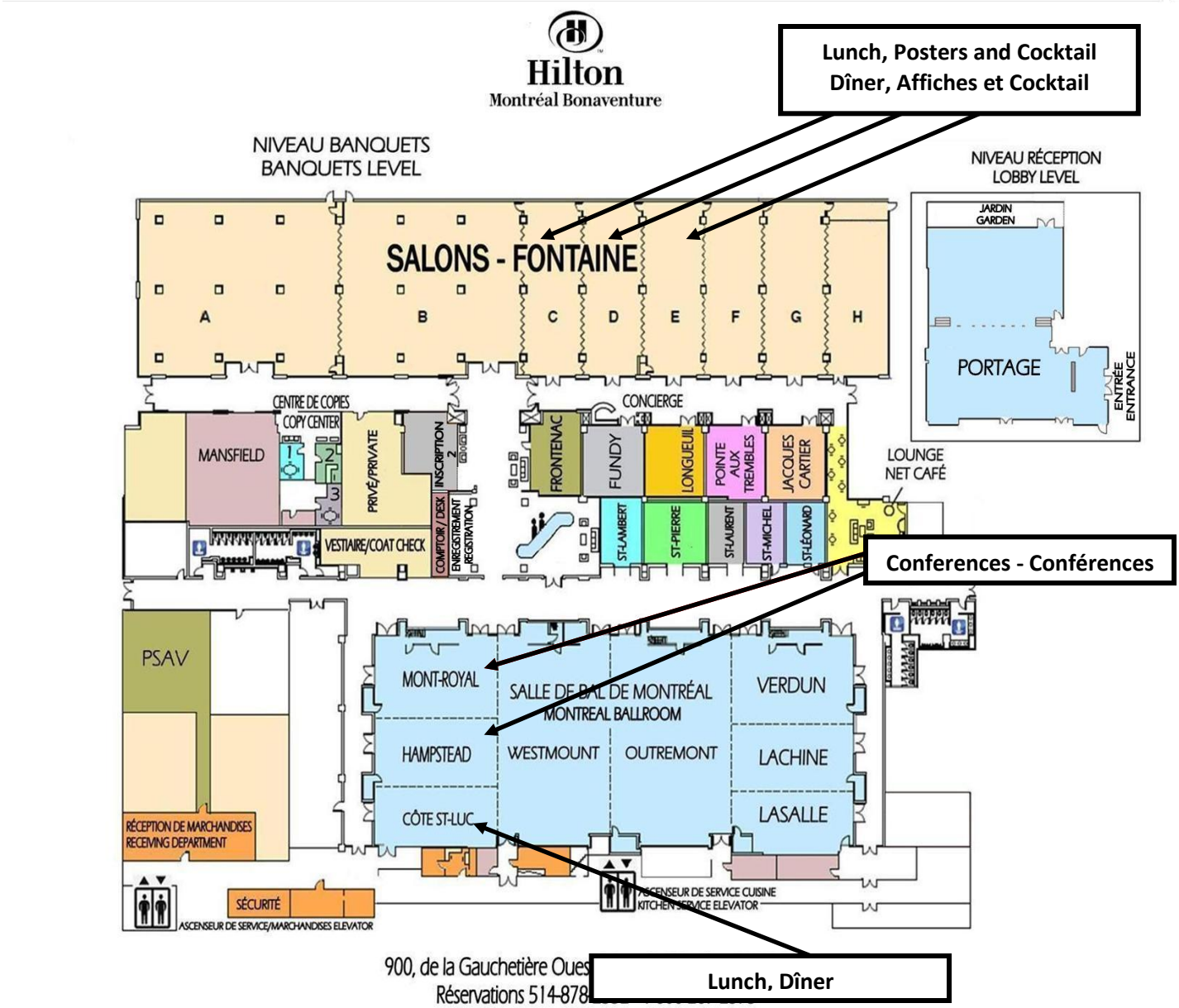
Floor Plan – Plan de l'étage



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