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

2nd Canadian Symposium on Hepatitis C Virus

2ème Symposium canadien sur le virus de l'hépatite C

March 4, 2013 – 4 mars 2013

Inn at Laurel Point, Victoria, BC

Program and Abstracts
Programme et résumés

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Welcome Message

Dear colleagues,

We would like to welcome you to the 2nd 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 people with HIV, people who inject drugs, and First Nations people.

Despite this internationally recognized success, it is evident that interactions between Canadian scientists, clinicians and the affected community need to be strengthened in order to effectively respond to current and future challenges in the management of the disease. We believe that the Canadian HCV conference provides an ideal forum to exchange research findings, promote collaboration, and create synergy among Canadian researchers, practitioners and people living with HCV. We hope that this second annual symposium will build on the success of the first meeting and continue to foster knowledge translation for researchers, healthcare practitioners and community-based groups working in the field of HCV.

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. We are pleased that we are able to continue to contribute to the organization of this important meeting.

We would like to welcome you to this meeting and to the beautiful city of Victoria. 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 Victoria. 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

Accreditation

Accredited by UBC CPD

CONTINUING PROFESSIONAL DEVELOPMENT
FACULTY OF MEDICINE

This event is an Accredited Group Learning Activity eligible for up to 7.25 Section 1 credits as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada. This program has been reviewed and approved by UBC Division of Continuing Professional Development.

Each physician should claim only those credits he/she actually spent in the activity.

Program – Programme – Spirit Rooms

07h00 - 08h00 Registration, Exhibition and Poster Area opens - Terrace Ballroom

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

Epidemiology and Public Health
Chairs: Dr. Julie Bruneau and Dr. Gregory Dore

08h15 - 08h45 Population-Based Strategies for HCV Testing
Dr. Bryce Smith, Centers for Disease Control and Prevention, Atlanta, USA
08h45 - 09h00 Hepatitis C Treatment Cost Effectiveness: A Synthesis of Available Data
Dr. Murray Krahn, University Health Network, Toronto, Canada
09h00 - 09h15 Increased Reported Rates of HCV in Canadian "Baby Boomers": Results of a 20 Year Cohort Analysis of Nationally Reported Data
Maxim Trubnikov, PHAC, Ottawa, Canada
09h15 - 09h30 Physician Practices for Liver Fibrosis Assessment in Chronic Hepatitis C: A Nationwide Canadian Survey
Giada Sebastiani, McGill University, Montréal, Canada

09h30 – 10h00 Coffee Break - Spirit Room Foyer

Clinical Sciences
Chairs: Dr. Marc Bilodeau and Dr. Mel Krajden

10h00 - 10h15 The End of Interferon: Direct-Acting Antiviral Therapy for HCV
Dr. Jordan Feld, University Health Network, Toronto, Canada
10h15 - 10h45 Clinical Interventions Toward the Eradication of HCV
Dr. Greg Dore, The Kirby Institute, University of New South Wales, Sydney, Australia
10h45 - 11h00 IL28B Plasma Level Correlated with Differential Expression of Interferon-Stimulated Genes during Chronically Evolving Acute HCV
Marion Depla, CR-CHUM, Montréal, Canada
11h00 - 11h15 TMC435 in Combination with Peginterferon and Ribavirin in Treatment-Naïve HCV Genotype 1 Patients: Final Analysis of the PILLAR Phase IIb Study
Morris Sherman, Toronto Western Hospital, Toronto, Canada

Debate: Testing and Screening for Hepatitis C Virus Infection: Should We Screen Everyone Born Between 1945-1965?
11h15 - 11h30 Pro: **Dr. Bryce Smith,** Centers for Disease Control and Prevention, Atlanta, USA
11h30 - 11h45 Con: **Dr. Greg Dore,** The Kirby Institute, University of New South Wales, Sydney, Australia
11h45 - 12h00 Hepatitis C Screening Approaches in Canadian Populations: The Evidence
Dr. Tom Wong, Public Health Agency of Canada, Ottawa, Canada
12h00 - 12h45 Panel discussion: Moderated by Dr. Jordan Feld
Dr. Bryce Smith, Dr. Greg Dore, Dr. Tom Wong, and Community Representatives

12h45 - 14h00 Lunch - Terrace Ballroom

Behavioural Sciences
Chairs: Dr. Gail Butt and Dr. Gerry Mugford

14h00 - 14h30 Mapping the Social Aspects of Hepatitis C Among People who Inject Drugs
Professor Tim Rhodes, Centre for Research on Drugs and Health Behaviour, London School of Hygiene and Tropical Medicine, London, UK

14h30 - 14h45 "It's Good That The Group is Here" – An Evaluation of a Psycho-Social Group to Support Engagement with HCV Treatment
Sanjeev Sockalingam, University Health Network, Toronto, Canada

14h45 - 15h15 Coffee Break - Spirit Room Foyer

Biomedical Sciences
Chairs: Dr. Matthias Götte and Dr. Thomas Michalak

15h15 - 15h45 Pathogenesis and Vaccine Development for HCV Infection
Dr. Takaji Wakita, National Institute of Infectious Diseases, Shinjuku, Japan

15h45 - 16h00 Vaccinating Canadians Against Hepatitis C
Dr. Michael Houghton, University of Alberta, Alberta, Canada

16h00 - 16h15 Biochemical Characterization of Genotype Dependent, Natural Resistance to Non-Nucleoside Inhibitors (NNIs) of HCV NS5B
Anupriya Kulkarni, McGill University, Montréal, Canada

16h15 - 16h30 Study of The Genetic Bottleneck During Mother-To-Child Transmission of Hepatitis C Virus
Sebastien Fauteux-Daniel, Research Center CHU Sainte-Justine, Montréal, Canada

16h30 - 16h45 Analysis of a Protective Immune Response During Multiple Episodes of HCV Infection
Mohamed Abdel-Hakeem, Université de Montréal, Montréal, Canada

16h45 - 17h00 Hepatitis C Virus Induced Up-Regulation of microRNA-27 Expression Promotes Hepatic Triglyceride Accumulation
Ragunath Singaravelu, University of Ottawa, Ottawa, Canada

17h00 - 17h15 Closing Remarks
Dr. Mel Krajden, Univ. of British Columbia, Vancouver, Canada

17h15 - 18h30 Cocktail and Poster viewing - Terrace Ballroom

Committees – Comités

Organizing Committee - Comité organisateur

Frank Bialystok, University of Toronto
Marc Bilodeau, Université de Montréal
Benedikt Fischer, Simon Fraser University
Jason Grebely, University of New South Wales
Michael Houghton, University of Alberta
Mel Krajden, University of British Columbia (Chair)
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Eve Roberts, University of Toronto
Selena M. Sagan, Stanford University
Lorne Tyrrell, University of Alberta
Tom Wong, Public Health Agency of Canada
Joyce Wilson, University of Saskatchewan

Abstract Reviewers - Réviseurs des résumés

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Julie Bruneau, Université de Montréal
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Benedikt Fischer, Simon Fraser University
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Naglaa Shoukry, Université de Montréal
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Session Chairs - Modérateurs de sessions

Marc Bilodeau, Université de Montréal
Julie Bruneau, Université de Montréal
Gail Butt, University of British Columbia
Gregory Dore, University of New South Wales
Matthias Götte, McGill University
Mel Krajden, University of British Columbia (Chair)
Thomas Michalak, Memorial University
Gerry Mugford, Memorial University

Speakers Biographies and Abstracts – Biographies des conférenciers et résumés

Epidemiology and Public Health

Dr. Bryce Smith, Centers for Disease Control and Prevention, Atlanta, USA

Biography

Bryce D. Smith, Ph.D., is a Lead Health Scientist at the Centers for Disease Control and Prevention in the Division of Viral Hepatitis and currently serves as the Lead for the Prevention Research and Evaluation Team. Dr. Smith has been in the Division for four years and his research has concentrated on hepatitis C prevention issues including screening interventions and policy, economic analyses, rapid testing, primary and secondary prevention with persons who inject drugs, and health services research. Dr. Smith recently led laboratory and field evaluations of HCV rapid point of care tests and is the lead author on the *Recommendations for the Identification Chronic Hepatitis C Virus Infection Among Persons Born during 1945–1965*. Prior to working in hepatitis, he was in the Division of HIV/AIDS Prevention at CDC where he conducted multi-site effectiveness evaluations of evidence-based interventions in community based settings.

Abstract

Population-Based Strategies for HCV Testing

In the United States, 3.2 million persons are living with HCV infection. In 2007, HCV-related deaths surpassed those from HIV, and HCV-associated morbidity is on the rise. Since 1998, CDC has recommended HCV testing based on transmission risks and medical indications such as injection drug use, blood transfusions and elevated alanine aminotransferase levels. However, up to 75% of HCV-infected persons remain unaware of their infection. Risk-based strategies have limited effectiveness due to barriers such as suboptimal physician awareness and knowledge of testing indication, HCV natural history, test interpretation, care, and treatment. Studies have found low levels of HCV testing even when risk and medical indications data are collected and documented for insured patients in primary care settings. These low levels of testing create missed opportunities to prevent HCV-associated morbidity and mortality as currently available HCV therapies can clear (i.e., cure) HCV infection in > 70% of persons treated. Given the limitations of risk-based testing strategies, CDC issued a population-based recommendation for one-time testing of all persons born during 1945-1965 to improve identification of persons chronically infected with HCV and link them to appropriate care and treatment. While a few countries (e.g., France, Japan) have implemented population-based HCV testing strategies, this approach is novel in the US. This presentation will outline the rationale for the US HCV testing recommendations, discuss next steps for US implementation, and present similar population-based strategies for HCV testing conducted by other countries.

Dr. Murray Krahn, University Health Network, Toronto, Canada

Biography

Dr. Murray Krahn is the Director of THETA (Toronto Health Economics and Technology Assessment Collaborative), the F. Norman Hughes Chair in Pharmacoeconomics at the Faculty of Pharmacy, Professor in the Faculties of Medicine and Pharmacy, University of Toronto, Senior Scientist the Toronto General Research Institute and Adjunct Scientist at the Institute for Clinical Evaluative Sciences, Toronto. He is also an attending physician in the division of General Internal Medicine at the University Health Network, Toronto. Dr. Krahn's research program focuses on the use of decision analytic methods to examine health policy and health decision-making. His recent research includes the development of clinical policy models, disease-specific utility instruments, and use of large administrative datasets for developing longitudinal cost models. He is also interested in methods that integrate competing scientific paradigms in the evaluation of new drugs and technologies.

Abstract

We review recent publications describing the cost effectiveness of HCV screening and treatment. In addition, we will very briefly review decision making frameworks involving cost effectiveness analysis in order to clarify the role of cost effectiveness studies in decisions around drug reimbursement.

Clinical Sciences

Dr. Greg Dore, The Kirby Institute, University of New South Wales, Sydney, Australia

Biography

Professor Dore is Head, Viral Hepatitis Clinical Research Program, The Kirby Institute, The University of New South Wales, and Infectious Diseases Physician, St Vincent's Hospital, Sydney Australia. He has been involved in viral hepatitis and HIV epidemiological and clinical research, clinical care and public health policy for nearly 20 years. He has developed extensive national and international collaborations, and is internationally recognized in the areas of natural history of acute and chronic HCV infection, therapeutic strategies for acute and chronic HCV infection, and natural history and therapeutic strategies for HIV/viral hepatitis coinfection.

He has published 200 peer-reviewed publications including recent publications in *Nature Genetics*, *Gastroenterology*, *Hepatology*, and *J Hepatology*. He is editor of two books on hepatitis C. He holds large-scale ongoing public sector research funding from U.S. National Institutes for Health, Canadian Institutes for Health Research, and Australian National Health and Medical Research Council.

Professor Dore's leadership in clinical practice and research is demonstrated through his recent Presidency of the Australasian Society for HIV Medicine (ASHM), and membership of State and Commonwealth advisory committees on hepatitis C.

Abstract

Clinical Interventions Towards the Eradication of HCV

The advent of interferon-free direct acting antiviral (DAA) therapy should provide the foundation for greatly enhanced HCV treatment uptake. The anticipated availability of all oral, short duration (possibly 12 weeks), highly effective and well tolerated DAA regimens over the next 2 – 5 years also enhances the feasibility of HCV treatment as prevention. Preliminary mathematical modelling studies based on interferon and ribavirin therapy indicated that enhanced HCV treatment delivery to people who inject drugs (PWID) reduces HCV prevalence and incidence, and is cost-effective. Subsequent interferon-free DAA-based modelling demonstrated that a rapid scale-up of HCV therapy among PWID to relatively moderate treatment uptake levels would have considerable impact on HCV transmission. Other high-risk populations for consideration of HCV treatment as prevention initiatives are HIV-infected men who have sex with men (MSM), and incarcerated populations. However, several obstacles to broadly-based implementation of HCV treatment as prevention strategies will need to be overcome, including sub-optimal levels of HCV screening, limited infrastructure for effective delivery of HCV treatment to marginalised populations, and high DAA pricing.

Dr. Jordan Feld, University Health Network, Toronto, Canada

Biography

Dr. Feld graduated from medical school at the University of Toronto in 1997 and then completed residency programs in Internal Medicine and Gastroenterology. Following his clinical training, Dr. Feld completed a clinical research fellowship in hepatology and then spent 4 years doing clinical and laboratory research in the Liver Diseases Branch of the National Institutes of Health. He received a Masters of Public Health from Johns Hopkins University and has worked extensively abroad, maintaining a strong interest in international health.

Dr. Feld returned to Toronto to join the faculty of the University of Toronto as an Assistant Professor of Medicine and clinician-scientist based at the Toronto Western Hospital Liver Center and the Sandra Rotman Centre for Global Health. His laboratory focuses on understanding treatment non-response in hepatitis C infection and more broadly on understanding the antiviral immune response with the goal of developing new strategies for the treatment of viral hepatitis.

Abstract

Primer to the Future Treatment of HCV

The past few years have seen enormous progress in the treatment of chronic HCV infection. The introduction of the first direct-acting antivirals (DAAs) has been a major breakthrough and a huge advance over standard peginterferon and ribavirin therapy. However, telaprevir and boceprevir are clearly just the tip of the iceberg. The field is moving at breakneck speed with each new international meeting providing important new results and an ever-expanding list of novel therapies. Newer, better-tolerated DAAs are in development and hold promise for highly effective, potentially interferon-free therapy in the not too distant future. With options expanding, the challenge will be to determine the best combination of agents. There are pros and cons to each of the different classes of DAAs ranging from differences in antiviral potency and resistance profile to tolerability and drug-interactions. Although it would be nice to have a one treatment fits all paradigm, it is possible that therapy will need to be tailored for individual patient groups. Different therapeutic approaches will be discussed ranging from combination therapies including peginterferon to the promise but significant challenges of interferon-free regimens. Rather than review data from all the drugs in development, critical results will be highlighted to illustrate relevant points to develop a picture of the future of HCV therapy.

Dr. Tom Wong, Public Health Agency of Canada, Ottawa, Canada

Biography

Dr. Thomas Wong, MD, MPH, CCFP, FRCPC is the Director of Professional Guidelines and Public Health Practice Division, Centre for Communicable Diseases & Infection Control at the Public Health Agency of Canada. He was trained in family medicine, internal medicine, infectious diseases and public health at McGill, Harvard and Columbia. His scientific interests focus on HIV, sexually transmitted infections, hepatitis C, tuberculosis and other infectious diseases, especially among underserved populations. Dr. Wong has academic appointments at both the University of Ottawa and the University of Toronto. He is the Chair of the Expert Working Group on Canadian Guidelines for Sexually Transmitted Infections, Editor-in-Chief of the Canadian Guidelines on Sexually Transmitted Infections, Co-Chair of the Sexually Transmitted and Bloodborne Infection Task Group and Associate Editor of the Canadian TB Standards

Abstract

It has been estimated that as many as 75% of hepatitis C (HCV)-infected individuals in the United States are unaware of their infection. In comparison, with a universal health care system and hepatitis C prevention efforts across Canada, it is estimated that 21% of HCV infections are undiagnosed in Canada. This presentation will highlight the screening efforts to prevent and control HCV in Canada. Preliminary estimates regarding HCV in different Canadian birth cohorts will be discussed.

Behavioural Sciences

Dr. Tim Rhodes, School of Public Health Psychology, London School of Hygiene and Tropical Medicine, London, UK

Biography

Tim Rhodes is Professor in Public Health Sociology and Director of the Centre for Research on Drugs and Health Behaviour at the London School of Hygiene and Tropical Medicine (University of London), and Conjoint Professor of the Sociology of Health at the University of New South Wales. He leads a programme of mixed-method qualitative research focused on understanding how social environments shape the health harms linked to drug use. Current studies include qualitative longitudinal research investigating access to harm reduction among people who inject drugs in Kenya; and with Magdalena Harris, life history research on how people who inject drugs avoid hepatitis C. He has published widely on the social relations of HIV and hepatitis C risk among people who inject drugs. He is Editor-In-Chief of the *International Journal of Drug Policy*.

Abstract

The Social Relations of HCV Treatment Engagement

Tim Rhodes and Magdalena Harris

Background There is low uptake of hepatitis C (HCV) treatment among people who inject drugs. This presentation maps a sociological approach towards understanding the 'problem' of HCV treatment engagement. Rather than envisaging treatment engagement primarily in relation to the innovation and availability of treatment technology, or in relation to patient compliance and awareness, we map treatment engagement as a product of social condition. **Approach** After providing an outline for how social research can help unpack the interplay of social and structural factors mediating treatment engagement, we draw on qualitative research to focus on two key themes: how *narratives of treatment expectation and entitlement shape engagement*; and how provider *strategies to tame the adverse effects of treatment systems* seek to create enabling environments for treatment. **Findings** We argue that a cultural narrative of rationed treatment expectation among people who inject drugs connects with ideas of the drug user as a less than fully acceptable or deserving citizen of treatment. This combines with a common narrative envisaging HCV treatment as part of a process of recovery towards normalcy, which undermines engagement among those who currently inject drugs. While provider strategies to 'tame' systemic barriers to accessing treatment negotiate more flexible arrangements of treatment delivery, as well seek to build trust and agency among would-be patients, they have their limits in successfully bridging how cultural narratives surrounding the drug using patient ration treatment expectation, entitlement and trust. **Conclusions** This analysis illustrates how the problem of hepatitis C treatment engagement is a product of social relations. We highlight the need for interventions which foster community action and a sense of collective entitlement to treatment among people who inject drugs in combination with service provider efforts to maintain a momentum of systemic change.

Biomedical Sciences

Dr. Takaji Wakita, National Institute of Infectious Diseases, Shinjuku, Japan

Biography

Takaji Wakita was born in 1958 and received his medical degree in 1983 from Nagoya University, Nagoya, Japan. He was then trained in the clinic for 5 years. He obtained his PhD in Internal Medicine III at Nagoya University in 1992 for a thesis entitled "Detection of pre-C and core region mutants of hepatitis B virus in chronic hepatitis B carriers". He received postdoctoral training at Molecular Hepatology Laboratory (Dr. Jack Wands' Lab), Massachusetts General Hospital Cancer Center, Boston between 1992 and 1995. Work commenced on antisense inhibition of hepatitis C virus.

In 1995, he moved to the Department of Microbiology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, and then moved to Tokyo Metropolitan Institute for Neuroscience as a Principal Investigator in 1998. In 2006, he moved to the National Institute of Infectious Diseases, Tokyo, Japan, and was appointed as a Director of Department of Virology II. His major research interests include: Infection and replication models of hepatitis C virus, pathogenesis of hepatitis viruses, and novel therapeutics and vaccine development for hepatitis virus infections. He received the Hideyo Noguchi Memorial Medical Award in 2008, and Oda Prize (awarded by the Japanese Society of Hepatology) in 2009.

Abstract

Hepatitis C virus replication models and vaccine development

Hepatitis C virus (HCV) infection causes chronic liver diseases and is a worldwide health problem. Despite the increasing demand for the knowledge of viral replication and pathogenesis, detailed analysis of viral life cycle has been hampered by the lack of efficient viral culture system. We isolated full-length HCV cDNA of JFH-1 strain from a fulminant hepatitis patient. RNA transcripts from full length JFH-1 or J6 and JFH-1 chimeric constructs were transfected into Huh7 cells, and efficient replication of viral RNA and secretion of recombinant viral particles into culture medium was observed. We also developed cell culture adapted infectious HCV strains with different genotypes. HCV culture system has been used for the detailed analysis of HCV life cycle. We have found several important host factors involved in each virus propagation steps and performed mechanistic analysis. We also aimed to develop prophylaxis vaccines for HCV infection. Purified recombinant HCV particles were inactivated and injected into mice with adjuvant and its immunogenicity was compared with recombinant envelope proteins. Sera from immunized mice were collected and their neutralizing effects against HCV infection were examined *in vitro* and *in vivo*.

Dr. Michael Houghton, University of Alberta, Alberta, Canada

Biography

Michael Houghton, PhD, is the Li ka Shing Professor and holder of the Canada Excellence in Research Chair (CERC) in Virology within the Li Ka Shing Institute of Virology at the University of Alberta where he is focusing on research into viral hepatitis and other inflammatory diseases. Together with Dr. G. Kuo & Dr. Q-L. Choo at the Chiron Corporation and Dr. D. Bradley at the CDC in the USA, his laboratory at Chiron discovered the hepatitis C virus, developed blood tests and identified new drug targets for this virus as well as developing a vaccine for clinical development. His laboratory was also the first to molecularly characterize the viral hepatitis D genome and the human beta-interferon gene. He and colleagues have received numerous awards for their work on hepatitis C including the Clinical Lasker and Karl Landsteiner awards from the USA, the Robert Koch Medal from Germany and the Gold Medal from the Canadian Liver Disease Association. He is an author of more than 200 research publications.

Abstract

Vaccinating Canadians against Hepatitis C

Up to thousands of new HCV infections occur each year in Canada mainly in IVDU populations although healthcare workers, paramedics and police officers are also at some increased risk as are babies born to mothers with high viral load. Development of a partially-effective vaccine now appears feasible and most likely, will rely on the induction of broad cross-neutralising antibodies and cross-reactive cellular immune responses against the virus. Effective implementation could start with identifying and vaccinating young IVDUs seronegative for HCV & HIV in the major metropolitan areas of the country and would likely be more efficient and cost-effective than treating newly-infected IVDUs with interferon-alpha or with the emerging but very expensive drug combinations. Such a strategy is especially feasible in Canada since it can be built upon the successful experience of monitoring and intervention in some IVDU populations within the country and because of the relatively small target populations. In parallel, activities aimed at better definition of immune-correlates of protection in IVDU populations may result in a vaccine registration strategy being based on demonstration of immunogenicity and safety rather than being based on challenging efficacy trials within this population. Successful implementation will rely on advanced planning by a multi-disciplinary collaborative team involving vaccine producers, national IVDU support groups, public health agencies and regulators.

Oral Abstracts – résumés oraux

Epidemiology and Public Health

Oral presentation at 09h00

INCREASED REPORTED RATES OF HCV IN CANADIAN "BABY BOOMERS": RESULTS OF A 20 YEAR COHORT ANALYSIS OF NATIONALLY REPORTED DATA

Maxim Trubnikov [1], Jane Njihia [1], Ping Yan [1], Chris Archibald [1]
1. Public Health Agency of Canada/Centre for Communicable Diseases and Infection Control (Ottawa, Canada)

Background: Historical analysis of data has become a standard approach to identifying and describing the role of external factors in the distribution of diseases. Identifying birth cohorts potentially more affected by hepatitis C virus (HCV) infection morbidity may better focus public health activities and ensure cost-effectiveness of such interventions.

Purpose: The purpose of this analysis was to study the association of the birth year and reporting period with rates of reported HCV cases in the Canadian Notifiable Diseases Surveillance System (CNDSS).

Method: HCV cases with information on sex, age, year of report and jurisdiction reported to the Public Health Agency of Canada (PHAC) from 1991 through 2010 were extracted from CNDSS. Birth year of HCV cases was back calculated by using year of report and age at diagnosis. Sex-specific population rates for 5-year age groups born between 1921 and 1990 were calculated per 100,000 by dividing age and sex specific HCV cases reported over 5-year period by the corresponding estimates of age- and sex-specific populations in Canada. Reported rates for 5-year birth cohorts were log-logit transformed and underwent the "mean polish" procedure. Residuals from the "mean polish" were plotted against birth cohorts in MS Excel to estimate the presence of interaction between age and reporting period of HCV cases. Rate ratios by birth cohort for the original HCV reported rates and their 95% confidence intervals were calculated, with males and females born in 1941-1945 utilised as reference birth cohort. Three-factor linear regression model, including cohort, period and their interaction, was fit for log-logit transformed HCV rates and their residuals.

Result(s): Rate ratios, the direction and the magnitude of the associations between age-cohort and period variables and the reported rates of HCV were similar in male and female subsets of the data. Males born between 1946 and 1965 had 21 to 40% increased rates of HCV case reports, while females of the same birth cohort, accounted for 12 to 43% increase in the rates in comparison with the reference birth cohort (Table 1)

Conclusion(s): Our analysis suggests a "cohort effect" in HCV cases reported in Canada. Males and females born between 1946 and 1965 appear to have increased reported HCV rates across the 20 year period of analysis.

Funding source (f): Public Health Agency of Canada

Oral presentation at 09h15

PHYSICIAN PRACTICES FOR LIVER FIBROSIS ASSESSMENT IN CHRONIC HEPATITIS C: A NATIONWIDE CANADIAN SURVEY

Giada Sebastiani [1, 2], Peter Ghali [1], Phil Wong [1], Marina Klein [2], Marc Deschenes [1], Robert Myers [3]
1. Division of Gastroenterology, Royal Victoria Hospital, McGill University Health Centre (Montréal, Canada); 2. Department of Medicine, Division of Infectious Diseases/Chronic Viral Illness Service, McGill University Health Centre (Montréal, Canada); 3. Liver Unit, Division of Gastroenterology and Hepatology, Department of Medicine, University of Calgary (Calgary, Canada)

Background: Staging of liver fibrosis is of paramount importance for determining prognosis and guiding management of patients with hepatitis C virus (HCV). Canadian guidelines for the management of chronic hepatitis C recommend that all HCV patients undergo an assessment for the stage of liver fibrosis. Non-invasive tools for the staging of liver fibrosis have been proposed instead of liver biopsy, a costly and invasive procedure. According to the Canadian guidelines, serum biomarkers and transient elastography (Fibroscan; Echosens, Paris, France) can be used instead of liver biopsy to stage HCV-related liver fibrosis with acceptable levels of accuracy. However, adherence to HCV guidelines and implementation of non-invasive tools instead of liver biopsy among Canadian physicians are unknown.

Purpose: The aim of this study was to investigate practices of liver fibrosis assessment among physicians who manage HCV patients across Canada.

Method: Hepatologists, gastroenterologists, infectious diseases specialists and primary care physicians, either members of the Canadian Gastroenterology Association or the Canadian HIV Trials Network, were invited to participate in this web-based, national survey.

Result(s): Among 237 invite physicians, 102 completed the survey (response rate 43%). Participation was 43% (102 respondents, 237 invitations). Overall, 80% of the respondents were male, 60% had age between 30 and 50 years, 81% were specialists in hepatology and/or gastroenterology, 71% dedicated more than 75% of their time to patients care, and 51% were based at a university hospital. The majority (77%) of the respondents requested assessment of liver fibrosis stage routinely in patients with HCV. Fibroscan was the main tool for liver fibrosis staging for 53% of the respondents, liver biopsy for 39% and Fibrotest-Fibrosure for 8.8%. For most of the respondents (61%) available non-invasive tools for liver fibrosis assessment had impacted their practice by reducing their performance of liver biopsy by at least 25%. The main concerns of respondents regarding non-invasive fibrosis tools were access/availability (in 42%), lack of guidelines for clinical use (27%) and cost/lack of reimbursement (14%). Overall, 38% of the respondents had a Fibroscan in their department. Of the remaining 62% of physicians only 40% had a convenient access to it. All respondents who neither had Fibroscan or convenient access to it would implement its use in clinical practice and reduce their reliance on liver biopsies if access was improved. Figure 1 depicts the characteristics of the respondents associated with higher use of non-invasive tools.

Conclusion(s): This nationwide survey shows that most of Canadian physicians who manage patients with HCV adhere to current guidelines regarding the routine assessment of liver fibrosis. Although biopsy remains the primary tool for fibrosis assessment for 40% of the respondents, non-invasive tools, particularly Fibroscan, have reduced the frequency of liver biopsy. Non-invasive tools for staging liver fibrosis, particularly Fibroscan, have significantly reduced the need for liver biopsy in Canada. Limitations in access/availability of the non-invasive tools represent a significant barrier. Finally, this study emphasizes the need for clinical guidelines and a better reimbursement policy in order to implement non-invasive fibrosis tools, which ultimately will minimize costs and invasiveness of liver biopsy.

Clinical Sciences

Oral presentation at 10h45

IL28B PLASMA LEVEL CORRELATED WITH DIFFERENTIAL EXPRESSION OF INTERFERON-STIMULATED GENES DURING CHRONICALLY EVOLVING ACUTE HCV

Marion Depla [1], Camille Brunaud [1, 2], Julie Bruneau [1, 3], Naglaa Shoukry [1, 3]
1. Centre de Recherche du CHUM, Hôpital St-Luc (Montréal, Canada); 2. Département de microbiologie et immunologie, Faculté de Médecine, Université de Montréal (Montréal, Canada); 3. Département de Médecine Familiale, Faculté de médecine, Université de Montréal (Montréal, Canada)

Background: Polymorphisms in the IL28B gene, encoding IFN-λ3, can predict spontaneous resolution of HCV. This predictive feature may be enhanced when combined with polymorphisms in the killer cell immunoglobulin-like receptor (KIR) genes controlling the function of natural killer (NK) cells. We have previously demonstrated that NK cells are activated during acute HCV infection irrespective of its outcome and that their activity correlated with the magnitude of adaptive T cell responses, essential for viral clearance. This suggests that cross-talk between the innate and adaptive components of the immune system is an important determinant of infectious outcome.

Purpose: We hypothesized that IL28B polymorphisms modulate the expression of IL28B during acute HCV and, consequently, the induction and cross-talk between innate and adaptive immunity including the induction of innate immune genes, activation and recruitment of NK cells and T cells to the liver.

Method: We performed longitudinal quantification of the plasma levels of IL28B by ELISA on plasma samples collected from a cohort of injection drug users (n=30) during acute HCV infection with different outcomes. In addition, we used qRT-PCR to monitor expression of seven genes previously associated with response to interferon following HCV infection in in vitro models and NK cell activity (IFI6, IFIT1, Mx1, USP18, IP-10, NCR3, KLRD1).

Result(s): We observed that patient homozygous for the favourable IL28B rs12979860 C allele (C/C) expressed higher levels of IL28B during early acute infection as compared to others with the non-favourable T allele (*/T) (p=0.0205). This observation became more significant when only acute infected patients with chronic evolution were analysed (p=0.0047). Higher IL28B plasma levels (above the median) correlated with decreased expression of IP-10 (p=0.0008), KLRD1 (CD94) (p=0.0021), IFIT1 (p=0.0052) and USP18 (p=0.0418) and increased expression of Mx1 (p=0.0077) only in patients with chronic evolution despite having the favourable genotype. In contrast, in patients having the unfavourable genotype and undergoing chronic evolution, IL28B plasma levels correlated with decreased expression of Mx1 (p=0.0062).

Conclusion(s): Our results suggest that IL28B polymorphism may modulate the recruitment of immune cells to the liver and the NK cell activity by affecting the expression of the interferon-stimulated genes IFIT1, USP18 and the chemokine IP-10, the antiviral protein Mx1, and the NK cell inhibitory receptor KLRD1.

Oral presentation at 11h00

TMC435 IN COMBINATION WITH PEGINTERFERON AND RIBAVIRIN IN TREATMENT-NAÏVE HCV GENOTYPE 1 PATIENTS: FINAL ANALYSIS OF THE PILLAR PHASEIIB STUDY

Michael Fried [1], Morris Sherman [2], Gregory Dore [3], Robert Flisiak [4], Peter Ferenci [5], Ira Jacobson [6], Patrick Marcellin [7], Michael Manns [8], Fred Poodard [9], Marie Beaumont-Mauviel [10]
1. University of North Carolina (Chapel Hill, United States); 2. Toronto Western Hospital (Toronto, Canada); 3. University of New South Wales (Sydney, Australia); 4. Medical University of Bialystok (Bialystok, Poland); 5. Allgemeines Krankenhaus der Stadt (Vienna, Austria); 6. Weill Cornell Medical College (New York, United States); 7. Hopital Beaujon Clichy (Paris, France); 8. Medizinische Hochschule Hannover (Hannover, Germany); 9. Cedars-Sinai Medical Center (Los Angeles, United States); 10. Tibotec BVBA (Beerse, Belgium)

Background: TMC435 is an investigational, once daily (QD), oral HCV NS3/4A protease inhibitor. PILLAR (TMC435-C205; NCT00882908) is an international, Phase IIb, randomized, double-blind study in treatment-naïve patients chronically infected with HCV genotype 1.

Purpose: The purpose of the PILLAR study was to assess efficacy and safety of TMC435 in combination with peginterferon α-2a/ribavirin (PegIFN/RBV).

Method: Patients were randomized to receive TMC435 (75 or 150 mg QD) for 12 or 24 weeks with PegIFN (180 g/wk)/RBV (1000–1200 mg/day), or placebo/PegIFN/RBV. In TMC435 arms, total PegIFN/RBV treatment duration was 24 or 48 weeks (24 if HCV RNA <25 IU/mL detectable/undetectable Week 4 and <25 IU/mL undetectable Weeks 12, 16, and 20). In the control arm all patients were treated for 48 weeks. Primary endpoint was SVR at Week 72.

Result(s): In TMC435 arms, RVR was achieved by 68-76% of patients (placebo=5%), SVR Wk72 was achieved by 71-85% (placebo=65%), and 79-86% were eligible to complete treatment at Week 24. Viral relapse occurred in 8-19% with TMC435 and 18% with placebo. Incidence of discontinuations, adverse events (AEs, including rash, anemia, and neutropenia) and serious AEs were similar in TMC435 and control arms. Mild, transient increases in direct and indirect bilirubin, not associated with increases in other hepatic parameters, were observed with TMC435 150 mg.

Conclusion(s): TMC435 75 or 150 mg QD in combination with PegIFN/RBV resulted in higher SVR rates compared with PegIFN/RBV alone, with a 24-week total treatment duration in the majority of patients, and a favorable safety profile. TMC435 150 mg QD is being studied in Phase III trials.

Funding source (f): Janssen

Behavioral Sciences

Oral presentation at 14h30

"IT'S GOOD THAT THE GROUP IS HERE" – AN EVALUATION OF A PSYCHO-SOCIAL GROUP TO SUPPORT ENGAGEMENT WITH HCV TREATMENT

Sanjeev Sockalingam [1, 2, 3], Zoë Dodd [4], Christopher Meaney [5], Kate Mason [4], Robert McKay [6]
1. Toronto Community Hepatitis C Program (Toronto, Canada); 2. University Health Network (Toronto, Canada); 3. University of Toronto (Toronto, Canada);
4. Toronto Community Hepatitis C Program, South Riverdale Community Health Centre (Toronto, Canada); 5. Department of Family & Community Medicine, University of Toronto (Toronto, Canada); 6. Toronto Community Hepatitis C Program, Sherbourne Health Centre (Toronto, Canada)

Background: Despite the emergence of more flexible, interdisciplinary models of Hepatitis C (HCV) treatment, uptake remains generally low amongst those populations most affected by the virus. More information is needed about the role and key components of psycho-educational support group to support HCV treatment and engagement, a common component for many of these programs.

Purpose: To determine the effectiveness of an HCV psycho-social support group intervention. We sought to examine which client factors affected drop-out/attendance, whether or not measures of group process improved over time and if either was related to positive psycho-social outcomes.

Method: The Toronto Community Hepatitis C Program (TCHCP) is a community-based model of HCV treatment offering weekly group support designed to improve access to HCV treatment for people who are active substance users and/or have serious mental health issues. Data was collected at baseline and at four subsequent time points per group cycle, over three cycles at each of the 3 program sites. Each group lasted 18 weeks with up to 20 participants, some of whom participated in multiple group cycles. Levels of depression and anxiety were measured by the PHQ 9 and GAD-7. Clinical group process measures were the Empathy Scale – Patient's Version and the TFI: Cohesiveness Scale. Demographics and health information were collected via chart review.

Analysis of factors potentially influencing drop outs and cumulative sessions attended were performed using logistic and linear regression, respectively. Comparison of PHQ 9 and GAD 7 scores between baseline and week 16 (in each cycle separately) was accomplished using a Wilcoxon Signed Rank test. Changes in empathy and group cohesion were assessed using F-tests of the time effect from linear mixed models.

Result(s): 163 participants attended during the study period. Of these, 91 were unique individuals: 43 participated in only one group cycle, 24 in two and 24 in three. 71% were male with an average age of 47 years. 75% were HCV genotype 1 and 10% were HIV co-infected. Substance use and mental health issues were common with 57% reporting current crack use and 47% a history of mental health related hospitalization. 10 people were on treatment when the study began and 10 more started during the study period. Average number sessions attended per cycle was 11 (61%). No demographic variables predicted group drop-out. Number of sessions attended was also not associated with any variable consistently over the 3 study cycles. Preliminary findings suggest that group cohesiveness is strong. Where both group process measures improved over time, measures of depression and anxiety also improved.

Conclusion(s): Attendance was higher than might be anticipated for this highly marginalized population. Group cohesion measures were equivalent or higher compared to norms for other psycho-educational support groups where there are fewer commodities. This study suggests that marginalized individuals living with HCV are able to engage and benefit from group therapy.
Funding source (f): This study was funded by South Riverdale Community Health Centre.

Biomedical Sciences

Oral presentation at 16h00

BIOCHEMICAL CHARACTERIZATION OF GENOTYPE DEPENDENT, NATURAL RESISTANCE TO NON-NUCLEOSIDE INHIBITORS (NNIS) OF HCV NS5B

Anupriya Kulkarni [1], Jean Bernatchez [1], Matthias Gotte [1]
1. McGill University (Montréal, Canada)

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.

Method: The HCV NS5B polymerase from different genotypes was expressed and purified, and the inhibitory activity of acyl pyrrolidine and 1,5-benzodiazepine was tested on them 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.

Result(s): 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. Using in silico analysis 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. By site directed mutagenesis, when introduced in NS5B 1b, these mutations showed decreased susceptibility when compared with the wild type. Specifically, we found that while mutants A218S and V405I showed significant resistance to both acyl pyrrolidine and 1,5-benzodiazepine, mutants A450S and C451T show borderline resistance.

Conclusion(s): 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 source (f): Canadian Institute of Health Research (CIHR), National CIHR Research Training Program in Hepatitis C (NCRTP).

Oral presentation at 16h15

STUDY OF THE GENETIC BOTTLENECK DURING MOTHER-TO-CHILD TRANSMISSION OF HEPATITIS C VIRUS

Sebastien Fauteux-Daniel [1, 2], Ariane Larouche [1, 2], Chanel Beland [1, 3], Normand Lapointe [4, 5], Valérie Lamarre [4, 5], Marc Boucher [5, 6], Deborah Money [7], Armelle Le Campion [1, 2], Jonathan Boulais [4], Hugo Soudeyns [1, 2]
1. Unité d'immunopathologie virale, Centre de recherche du CHU Sainte-Justine (Montréal, Canada); 2. Département de microbiologie et immunologie, Université de Montréal (Montréal, Canada); 3. Programme des sciences biomédicales, Université de Montréal (Montréal, Canada); 4. Département de pédiatrie, Université de Montréal (Montréal, Canada); 5. Centre maternel et infantile sur le SIDA, Centre de recherche du CHU Sainte-Justine (Montréal, Canada); 6. Département de gynéco-obstétrique, Université de Montréal (Montréal, Canada); 7. Women's Health Research Institute (WHRI) (Vancouver, Canada)

Background: Mother-to-child transmission (MTCT) of hepatitis C virus (HCV) is the main route of infection for children in developed countries. However, the underlying biological mechanisms involved in MTCT of HCV are unclear, and the reasons that underpin its relative inefficacy (<10%) remain obscure1. Like other RNA viruses, HCV is found in its host as a family of closely related variants called a quasispecies2, 3. A large share of HCV sequence variation is concentrated in hypervariable regions (HVR) of the E2 envelope gene, including HVR1, 2, and 3. HVRs are subjected to selective pressure exerted by host humoral immune response4. When HCV is transmitted by contact with contaminated biological fluids, only a small fraction of the quasispecies is transmitted, a phenomenon called a « transmission bottleneck »5.

Purpose: The main objectives of this project are: a) to determine whether a transmission bottleneck exists in MTCT of HCV; and b) to characterize this bottleneck using high-definition sequence-based analysis. These experiments will allow us to test whether or not the transmission bottleneck is related to HCV-specific humoral immune responses in the mother and/or the child.

Method: The HCV quasispecies profile based on the sequence of the E2 envelope protein gene was studied in blood samples obtained from 3 mother/child dyads. HCV-infected mothers were tested at the time of delivery. HCV-infected children were tested at 1 month and 6 months of age. Study subjects were enrolled at CHU Sainte-Justine (Montreal) and WHRI (Vancouver). HVRs were amplified using barcode-tagged primer sets and high-fidelity reverse transcriptase and polymerase preparations. PCR products were sequenced on a Roche 454 GS-FLX System using Titanium series reagents (Genome Quebec, Montreal). Sequencing datasets were processed using the MEGA 5 software package6. Amplification and sequencing error frequencies were assessed by processing plasmid-derived amplicons. The presence of HCV-neutralizing antibodies in the mother and/or her infant was tested by infecting Huh7.5 cells with RSV-based HCV pseudoparticles (HCVpp)7 bearing autologous E2 envelope segments and a luciferase reporter gene in presence of serial dilutions of maternal or infant serum samples.

Result(s): An average of 4794 HVR sequences were obtained per sample/time point. The average number of distinct viral variants isolated from the mothers was 79 (range=23-122), while the average number of viral variants isolated from the children at the first and second time points was 64 (range=42-101) and 55 (range=43-78) respectively. There were between 0 and 24 shared variants between the mother and the first time point tested in the child, and an average of 49 shared variants (range=28-79) between the first and second time point in the child.

Conclusion(s): Results of HCV quasispecies pyrosequencing indicate that MTCT of HCV may involve transmission of numbers of viral variants ranging between 1 to 24, a situation that appears to be singularly different from that observed in MTCT of human immunodeficiency virus type 1 (HIV-1)9. Taken together, these results yield an unprecedentedly-detailed portrait of the HCV transmission bottleneck and will enable us to directly test the involvement of humoral immune responses in this process.

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Funding source (f): Canadian Institutes of Health Research (to HS, NL, VL, and MB).

Oral presentation at 16h30

ANALYSIS OF A PROTECTIVE IMMUNE RESPONSE DURING MULTIPLE EPISODES OF HCV INFECTION

Mohamed Abdel-Hakeem [1, 2], Nathalie Bédard [1], Julie Bruneau [1, 3], Naglaa Shoukry [1, 3]
1. Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Hôpital St-Luc (Montréal, Canada); 2. Département de Microbiologie et immunologie (Montréal, Canada); 3. Département de médecine familiale, Université de Montréal (Montréal, Canada)

Background: Adaptive CD4 and CD8 T cell immune responses are essential for spontaneous clearance of hepatitis C virus (HCV) infection. However, the effective design of a prophylactic vaccine against HCV has been hampered by the limited knowledge of the functional signatures of a protective immune response upon re-exposure in real life settings. Furthermore, the complementary roles of cellular and humoral immunity in protection against re-infection remain elusive.

Purpose: To identify the signature of a protective immune response against HCV and define its determinants; with respect to its magnitude, breadth and Quality (phenotype and functionality).

Method: We performed detailed longitudinal analysis of the immune response in a cohort of intravenous drug users (n=10) during multiple episodes of HCV infections with different outcomes. We have compared the immune response in 6/10 patients who spontaneously resolved two successive infections and 4/10 who failed to clear the second infection.

Result(s): In patients who spontaneously resolved two successive infections, we observed a 2-19 fold expansion in HCV-specific CD4+ and CD8+ memory T cells upon reinfection, as measured by ELISPOT, with an increase in the breadth of response. Studies using MHC Class I tetramers demonstrated an expansion as high as 100 fold in HCV-specific memory CD8+ T cell with the specific appearance of a CD127 negative population at the peak of the response that rapidly disappeared upon viral clearance. In addition, we observed shifting epitope dominance in one of the studied patients. In contrast, HCV-specific memory T cells did not expand in patients who failed to clear the second infection or upregulated the exhaustion marker PD-1. The functionality of HCV-specific immune cells, viral sequences and humoral immune responses are under investigation.

Conclusion(s): This is the first study to dissect the characteristics of the adaptive immune response during multiple episodes of HCV infection in humans. The preliminary results suggest that protection from persistence upon reinfection with HCV is associated with a pronounced expansion in HCV-specific memory response and an increase in the breadth of response.

Funding source (f): * This work is supported by grants from the Canadian Institutes for Health Research (CIHR), Canadian Excellence Research Chairs (CERC) and the Fonds de recherche du Québec - Santé (FRQS). Mohamed Abdel–Hakeem is the recipient of doctoral fellowships from CIHR, the National CIHR Research Training Program on Hepatitis C (NCRTP-Hep C) and Université de Montréal.

Oral presentation at 16h45

HEPATITIS C VIRUS INDUCED UP-REGULATION OF MICRORNA-27 EXPRESSION PROMOTES HEPATIC TRIGLYCERIDE ACCUMULATION

Ragunath Singaravelu [1, 2], Ran Chen [3, 4], Rodney Lyn [5, 2], Daniel Jones [6], Rouleau Yanouchka [2], Shifawn O'Hara [2], Jenny Cheng [2], Rodney Russell [6], Lorne Tyrrell [3, 4], John Pezacki [7, 2]
1. University of Ottawa (Ottawa, Canada); 2. National Research Council of Canada (Ottawa, Canada); 3. Department of Medical Microbiology and Immunology, University of Alberta (Edmonton, Canada); 4. Li Ka Shing Institute of Virology, Katz Centre for Pharmacy and Health Research (Edmonton, Canada); 5. Department of Chemistry, University of Ottawa (Ottawa, Canada); 6. Immunology and Infectious Diseases, Memorial University of Newfoundland (St. John's, Canada); 7. Department of Biochemistry, Microbiology and Immunology and Department of Chemistry, University of Ottawa (Ottawa, Canada)

Background: MicroRNAs (miRNAs) are small RNAs that post-transcriptionally regulate gene expression. Their aberrant expression is commonly linked with diseased states, including hepatitis C virus (HCV) infection. The biological relevance of these HCV-induced modulations in the host miRNA milieu isn't fully understood.

Purpose: The HCV lifecycle is intimately linked to host lipid metabolism, including the induction of intracellular lipid accumulation to facilitate pathogenesis. Clinically, this manifests as hepatic steatosis, which is prevalent in over ~40% of HCV infected patients. We believe that a subset of virus-induced alterations in miRNA expression may contribute to HCV's hijacking of host lipid homeostasis.

Result(s): Herein, we demonstrate that HCV replication induces the expression of miR-27 in cell culture and in vivo HCV infectious models. Furthermore, we establish that miR-27 overexpression in hepatoma cells results in hepatic triglyceride accumulation and larger lipid droplets, as observed by triglyceride assays and coherent anti-Stokes Raman scattering (CARS) microscopy. This triglyceride accumulation coincides with miR-27's repression of PPAR-α and ANGPTL3 mRNA levels, as measured by qRT-PCR. PPAR-α is a transcription factor which activates the expression of genes associated with fatty acid catabolism. We demonstrate that treatment with a PPAR-α agonist, bezafibrate, is able to reverse the miR-27 induced lipid accumulation in Huh7 cells. This miR-27 mediated repression of PPAR-α signaling represents a novel mechanism of HCV-induced hepatic steatosis. Lastly, we also determine that miR-27 overexpression inhibited viral replication in hepatoma cells stably expressing HCV full-length genomic replicon.

Conclusion(s): Collectively, our results highlight HCV induced expression of a non-coding small RNA, miR-27, as a novel potential mechanism of steatosis. Our study also demonstrates the anti-HCV therapeutic potential of miR-27.

Funding source (f): NSERC

Posters - Affiches

Epidemiology and Public Health

Poster number: 100

THE EFFECT OF PRIMARY CARE ACCESS ON SUBSEQUENT HUMAN IMMUNODEFICIENCY VIRUS AND HEPATITIS C VIRUS INFECTIONS AMONG INJECTION DRUG USERS

Andreea Adelina Artenie [1], Julie Bruneau [2, 3], Élise Roy [4, 5]
1. Department of Family Medicine, Faculty of Medicine, McGill University (Montréal, Canada); 2. Research Center, University of Montréal Hospital Center (Montréal, Canada); 3. Department of Family Medicine, Faculty of Medicine, University of Montréal (Montréal, Canada); 4. Addiction Research and Study Program, Faculty of Medicine and Health Sciences, University of Sherbrooke (Sherbrooke, Canada); 5. Montréal Public Health Department, Agency for Health and Social Services of Montréal (Montréal, Canada)

Background: Background: Transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) among injection drugs users (IDUs) is a growing global concern. In Canada, IDUs account for 13% and for the majority of new HIV and HCV infections, respectively. The significant spread of these blood borne viruses among IDUs is largely due to unsafe injection practices. Left untreated, both infections can lead to debilitating illnesses and premature mortality. Chronic HCV infections are now the leading national cause of liver transplantation. Additionally, IDUs face a myriad of other, potentially life-threatening medical complications. Yet, poor access to primary care results in this marginalized population over-relying on emergency departments and in frequently requiring hospitalizations. The costs associated with these medical interventions are significant. Since substance misuse is a chronic condition, primary care settings have been indirectly suggested to be an essential component in the management of these patients. In this context, IDUs could benefit from screening, counseling and treatment for HIV/HCV infections. This is crucial since, according to data gathered though the survUDI network, ~25% of IDUs found seropositve for HIV/HCV are not aware of being infected.

Purpose: The overall objective of the study is to assess the effect of primary health care exposure, measured by visits to a primary care physician, on subsequent HIV/HCV transmission among IDUs, aged 18 years and older, and living in Montreal. The specific objectives are: 1) To examine factors associated with primary health care exposure at baseline; and 2) To examine the association between primary health care exposure and HIV/HCV incidence i) at baseline and ii) on a regular basis.

Method: This project will be conducted using the St. Luc/HEPCO Cohort database, developed through a prospective open cohort (1988-) of IDUs. For this project, eligibility includes being over 18 years of age, residing in Montreal, having injected drugs within the past six months, and being seronegative for HIV/HCV at study entry. Participants are enrolled through street level recruitment, word-of-mouth referral and community programs. At each biannual interview, data are collected through a validated questionnaire, and presence of viral antibodies is determined using ELISA. The study population will be characterized using descriptive analyses. Logistic regression will be used to study factors associated with primary health care exposure. HIV and HCV incidence rates will be estimated assuming the exponential time to-event model, with constant hazard rate, and corresponding 95% Confidence Intervals will be calculated using the Poisson distribution. Multivariable Cox proportional hazards regression models will be estimated separately for HCV and HIV incidences. For this study, 350 and 900 participants will be available for the HIV and HCV analyses, respectively (80-90% power).

Conclusion(s): Although the ultimate goal of addiction treatment is "complete remission", a more immediate and achievable goal is to limit the detrimental consequences of this practice and therefore, diminish the associated individual and social harms, and costs. This project has the potential to provide evidence-based knowledge to support the development of innovative primary care models that would provide HIV/HCV screening, counseling and treatment to this underserved population.

Funding source (f): CIHR awarded to Dr. Julie Bruneau

Poster number: 101

THE DEVELOPMENT AND IMPLEMENTATION OF A MULTILINGUAL HEPATITIS C MEDIA AND EDUCATIONAL OUTREACH CAMPAIGN TARGETTING IMMIGRANT GROUPS IN ONTARIO

Hywel Tuscano [1], Jim Pollock [1], Ed Jackson [1], Jeff Rice [1], Fozia Tanveer [1]
1. CATIE (Toronto, Canada)

Background: CATIE is Canada's source for up-to-date, unbiased information about HIV and hepatitis C. The organization connects people living with HIV or hepatitis C, at-risk communities, healthcare providers and community organizations with the knowledge, resources and expertise to reduce transmission and improve quality of life.
CATIE developed an Ethnocultural Hepatitis C Outreach project that produced in-language hepatitis C resources and a media campaign for four major immigrant communities living in Ontario: Pakistani, Punjabi, Chinese and Filipino. This project, funded by the Ontario Ministry of Health and Long-term Care, is part of the province's strategy to address hepatitis C. The program was recently launched in the Toronto area with great support from a number of community-based organizations due to the disproportionate number of cases of hepatitis C among Canadian immigrants.

Purpose: Of all the hepatitis C infections reported in Canada, 21 per cent are estimated to be among immigrants. Immigrants also face cultural and linguistic barriers to healthcare, and in Canada immigrant health is shown to decline over time. This project aims to increase awareness of the high prevalence of hepatitis C among these immigrant populations, encourage them to be tested for the virus and help direct them to resources that will better prepare them to seek testing and treatment.

Method: A multi-level strategy including education, outreach and social marketing aims to raise awareness of hepatitis C within the four largest immigrant communities in Ontario. International prevalence rates, blood safety and immigrate rates were assessed to determine the four communities to work with in Ontario.
Development of the project was through partnership and community consultation including workshops on media literacy, immigrant health and hepatitis C. Capacity building activities included the training of six bilingual community facilitators who delivered workshops in English, Simplified Chinese, Punjabi, Tagalog and Urdu.
Medical and community reviewers from each immigrant population assisted with the translations.
An Ethnocultural marketing company, DiversiPro, worked with CATIE on a media campaign, "Hepatitis C. Learn More. Get Tested.", in print, web and radio public service announcements. The provincial AIDS and Sexual Health Infoline, run by Toronto Public Health, also partnered with the campaign to provide phone counseling for individuals and to refer them to testing sites.

Result(s): During the campaign's development, six workshops on media literacy were delivered to 128 people and eight workshops on immigrant health and hepatitis C were delivered to 118 people to inform the development of the campaign and resources.
The website, yourlanguage.hepcinfo.ca, launched in November, 2012. Within its first month online, the website received 3,422 unique visitors. The first wave of the social marketing campaign ran in 26 media outlets in November, 2012 and will run again in January, 2013.
Multilingual Hepatitis C pamphlets launched in December, 2012 are available free in Canada from CATIE's Ordering Centre. They are available in Simplified Chinese/English, Tagalog/English, Punjabi/English and Urdu/English. PDF versions are also available on the website.

Conclusion(s): The project's success, evident in the community response, uptake from media and visits to the website, within the first year and a half of its development can be attributed to meaningful partnership, community engagement and a strong interest to raise awareness of hepatitis C within immigrant communities. Evaluation of the project's activities including the educational workshops, media campaign and resources will strengthen the framework for ongoing hepatitis C work within immigrant communities (available April, 2013). The project is funded until March, 2014 and will expand available languages according to assessed need and continue delivering service-provider workshops.

Funding source (f): The Hepatitis C Secretariat, AIDS Bureau, Ontario Ministry of Health and Long Term Care.

Clinical Sciences

Poster number: 200

PERSISTENCE OF HCV DURING AND AFTER CLINICALLY APPARENT SUCESSFUL TREATMENT OF CHRONIC HEPATITIS C

Thomas I Michalak [1], Annie Y Chen [1], Marija Zeremski [2], Ira M Jacobson [2], Andrew H Talal [2, 3]
1. Molecular Virology and Hepatology Research Group, Faculty of Medicine, Memorial University (St. John's, Canada); 2. Center for the Study of Hepatitis C and Division of Gastroenterology and Hepatology, Weill Cornell Medical College (New York, United States); 3. State University of New York (Buffalo, United States)

Background: Resolution of chronic hepatitis C (CHC) following therapy with PEG-IFN/ribavirin (RBV) is considered when serum HCV RNA becomes repeatedly undetectable and liver enzymes normalize. However, long-term persistence of low levels of HCV RNA in plasma, lymphoid cells and liver has been reported when highly sensitive assays and testing of serial plasma and PBMC samples were applied.

Purpose: To re-analyze sequential plasma and PBMC samples from patients who resolved CHC and became HCV RNA negative by clinical laboratory testing.

Methods: Plasma samples (n=60) from 11 randomly selected patients who resolved CHC after a standard course of PEG/IFN/RBV therapy were collected before (n=12), during (range 48-68 wks; n=28) and up to 33.1 (range 12-88) wks post-treatment (n=20). PBMCs (n=26) from 4 patients before (n=5), during (n=13) and post-treatment (n=8) were also analyzed. Total RNA was extracted from 250 or 750 µl plasma and intact or PHA-stimulated PBMCs. HCV RNA was detected by RT-PCR/nucleic acid hybridization (RT-PCR/NAH; sensitivity <5 copies/µg RNA or <2 IU/ml) (Pham et al., JVI 2004; 78:5867). Clone sequence analysis of the HCV 5'-UTR from sequential plasma and PBMCs was done in 2 patients.

Results: HCV RNA was detected in 9 of 20 (45.3%) plasma and 4 of 8 (50%) PBMC samples for up to 23.6 wks (range 12-59 wks) after completion of treatment. Among plasma samples identified during therapy as negative for HCV RNA by clinical assay, 64.3% were reactive by RT-PCR/NAH. Testing of RNA from 750 µl plasma increased HCV detection from 31.7% to 63.3% (38/60) compared to 250-µl samples. Testing naïve versus PHA-stimulated PBMCs enhanced HCV detection from 26.9% to 69.2% (18/26). Virus replicative strand was detected in 12/18 PBMC samples. Mutations identified in the 5'-UTR sequence persisted in plasma and/or PBMCs during and after PEG-IFN/RBV therapy. The frequency of HCV detection tended to decline in both plasma and PBMCs with longer follow-up.

Conclusions: HCV can persist at levels not detectable by standard clinical assays in both plasma and PBMC after clinically apparent resolution of CHC due to PEG-IFN/RBV therapy. The findings suggest the need for continued evaluation even after patients achieve undetectable HCV RNA post-treatment.
Funding sources: CIHR operating grant MOP-77544.

Behavioral Sciences

Poster number: 300

PUBLIC HEALTH AND HUMAN RIGHTS IN PRISONS: LITIGATING FOR PRISON-BASED NEEDLE AND SYRINGE PROGRAMS

Sandra Ka Hon Chu [1], Annika Ollner [2], Richard Elliott [1], Seth Clarke [2], Steven Simons , Ed Jackson [3], Carrie Robinson [4], Walter Cavalieri [5], Zoe Dodd [6]
1. Canadian HIV/AIDS Legal Network (Toronto, Canada); 2. PASAN (Toronto, Canada); 3. CATIE (Toronto, Canada); 4. CAAN (Toronto, Canada); 5. Canadian Harm Reduction Network (Toronto, Canada); 6. East Toronto Hepatis C Program (Toronto, Canada)

Background: Almost 20 years after an expert committee acknowledged the need for prison-based needle and syringe programs (PNSPs) and despite mounting evidence in support of such programs and numerous requests from community organizations, PNSPs do not exist in Canada. Repeatedly, the Canadian government has denounced PNSPs and clung to a "zero tolerance policy" on drugs in prison. However, significant investments in drug interdiction initiatives have yielded little success and rates of drug use remain steady behind bars.

Purpose: Rates of HIV and hepatitis C virus (HCV) are at least 10 and 30 times higher in Canadian federal prisons than in the general population. Not only is this a public health concern, but a violation of prisoners' constitutional rights.

Method: Having advocated for PNSPs, with little success, before parliamentary committees, with public health and correctional officials, and through public education and media campaigns, a constitutional challenge was initiated by a former prisoner and four HIV organizations. The case will principally rest on the Canadian Charter of Rights and Freedoms, which protects prisoners' rights to life, liberty and security of the person and to equal treatment before and under the law, and equal protection and benefit of the law without discrimination.

Result(s): The various forms of advocacy undertaken before the launch of the constitutional challenge were instrumental in building public support for PNSPs and helped to identify an applicant who was infected with HCV while incarcerated. A first volume of materials in support of the challenge was filed in September 2012, complemented by a website and video campaign.

Conclusion(s): The applicants seek an order directing Canada's correctional service to ensure the implementation of PNSPs in federal correctional institutions, in accordance with professionally accepted standards.

Biomedical Sciences

Poster number: 400

HEPATITIS C VIRUS REQUIRES THE RECRUITMENT OF THE AUTOPHAGY ELONGATION COMPLEX (ATG5-12/16) AT THE REPLICATION SITE

Ahmed Fahmy [1], Christian Duguay [1], Patrick Labonté [1]
1. INRS-Institut Armand Frappier (Laval, Canada)

Background: Hepatitis C virus (HCV) is a global burden affecting over 200 million people around the world. Chronic infection leads to cirrhosis and hepatocellular carcinoma. The best therapy is a combination of pegylated interferon and ribavirin. However, this therapy is effective in 50% of cases. Consequently, the need for more effective treatments is still required. Autophagy is a process aimed at maintaining cellular homeostasis. Many studies have demonstrated that autophagy activation upon viral infection can enhance or limit viral spread. Recently, it was reported that HCV infection induces autophagy and triggers accumulation of autophagic vesicles as observed by the typical punctate cytoplasmic distribution of LC3-II in infected cells.We showed earlier that viral RNA dependent RNA polymerase (NS5B) interacts with ATG5, a major component of autophagy initiation.

Purpose: In this study, we evaluate the involvement of the autophagy elongation complex (ATG5-12/16) in HCV replication

Result(s): We demonstrate that the elongation complex is recruited at the site of viral replication and acts as a proviral factor. Indeed, ATG5-12 as well as ATG16L1 colocalizes with the viral replicase in infected cells. Furthermore, we show that induction of autophagy by NS4B results in LC3-II colocalization in transfected cells but not in infected cells. Interestingly, LC3-I is not recruited to the elongation complex at the site of viral replication and no sign of colocalization of LC3-II with viral proteins was observed. Finally, using dominant negative forms of ATG5 ATG12 and ATG4, we demonstrate that the ATG5-12 conjugate is important for viral replication but not LC3-II formation

Conclusion(s): Together, these findings indicate that HCV uses the autophagy elongation complex as a proviral factor for its own replication but blocks the formation of a genuine autophagosome at the site of viral replication.

Funding source (f): NCRTP-HepC, NSERC

Poster number: 401

HEPATITIS C VIRUS NS5A DOWN-REGULATES VIRAL TRANSLATION THROUGH A MECHANISM REQUIRING THE POLY-U/UC REGION IN THE 3'UNTRANSLATED REGION

Brett Hoffman [1, 2], Qiang Liu [2]
1. School of Public Health, University of Saskatchewan (Saskatoon, Canada); 2. Vaccine and Infectious Disease Organization, University of Saskatchewan (Saskatoon, Canada)

Background: Although known to be essential for HCV replication, the role NS5A plays in viral translation has been controversial. Contradictory studies have concluded that NS5A stimulates, inhibits or has no effect on viral translation using reporter systems which lack the 3'untrasnlated region (UTR) of HCV. However, the 3'UTR may be involved in modulating viral translation as we and others have shown that NS5A binds to the poly-U/UC region within the 3'UTR, which suggests the potential for modulatory effects on viral translation through protein-RNA interaction.

Purpose: We set out to determine the role of NS5A in modulating viral translation and the role the poly-U/UC region plays in this modulation.

Method: To investigate the effect of NS5A-RNA interaction on viral translation, we utilized a set of monocistronic RNA reporter constructs. These constructs contain the 5' and 3'UTRs, with or without the poly-U/UC region of HCV and an internal Renilla luciferase reporter gene. 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. These reporters were used in combination with an NS5A expression plasmid.

Result(s): NS5A was found to specifically down-regulate viral translation in a dose-dependent manner which requires the presence of the poly-U/UC region of the viral 3'UTR. A similar inhibitory effect of NS5A on viral translation was observed using an HCV genomic RNA. In addition, we determined that the three domains of NS5A are capable of modulating viral translation independently and we chose to further map the effector region of domain I. Our results suggest that a 61 aa region within domain I is sufficient for translation down-regulation. Furthermore, we showed that NS5B, the viral RdRp, negates NS5A down-regulation of viral translation, possibly by binding NS5A and sequestering it from binding to the 3'UTR. The cellular factor IGF2BP1, which enhances viral translation via binding to the poly-U/UC region, appears to be out-competed by NS5A for its effect on HCV translation.

Conclusion(s): These findings suggest that in addition to being essential for viral replication, NS5A has an important role in modulating viral translation through a mechanism requiring the poly-U/UC region of the viral 3'UTR.

Funding source (f): NSERC, NCRTP-HepC

Poster number: 402

HEPATITIS C VIRUS-INFECTED CELLS DOWN-REGULATE NKP30 AND INHIBIT EX VIVO NATURAL KILLER CELL FUNCTIONS

Kayla Holder [1], Staci Stapleton [1], Maureen Gallant [1], Rodney Russell [1], Michael Grant [1]
1. Immunology and Infectious Diseases, Memorial University of Newfoundland (St. John's, Canada)

Background: Persistent viral infection requires immune evasion and hepatitis C virus (HCV) accomplishes this in part by modulating natural killer (NK) cell functions. NK cells defend against some pathogens, without prior exposure, by recognizing and destroying infected and transformed cells. NK cell cytotoxic activity is tightly regulated by the balance between the stimulation of activating and inhibitory receptors. Ligands for NK receptors can be up- or down-regulated upon HCV-infection to inhibit NK cell functions and establish chronic infection.

Purpose: We find NK functions are inhibited ex vivo by short-term exposure to HCV-infected hepatoma (Huh-7.5) cells. Our hypothesis is that HCV-infected Huh-7.5 cells up-regulate cell surface receptors and interact with NKp30 to alter NK cell signaling and, as a consequence, NK cytotoxicity.

Method: Fresh peripheral blood mononuclear cells (PBMC) were incubated 5h with K562 or W6/32 (anti-class I MHC)-coated C1R-B27 cells, uninfected or HCV-infected Huh-7.5 cells or cell free HCV. Co-cultured NKs were analyzed for surface CD56, NKG2D, CD16, NKp30, and CD107a expression and for intracellular IFN-γ and TNF-α. Potential CD81/HCV E2 interactions were considered by pre-incubating PBMC with soluble α-CD81 or Huh-7.5 cells with α-HCV E2. HCV-infected Huh-7.5 HLA-E surface expression was assessed by flow cytometry.

Result(s): NK cytotoxicity and ADCC were reduced by a mean of 23% ±2.2% (12 donors, $p<0.0001$, Student's paired t test) and 18% ±6% (6 donors, $p=0.001$, Student's paired t test), respectively, in 5h co-culture with HCV-infected Huh-7.5 cells. Flow cytometry revealed a 20-34% decrease in NK CD107a expression, 36-47% and 15-18% decline in NK IFN-γ and TNF-α production, respectively. Cell-free virus had no effect on NK cells and trans-well assays indicate cell-to-cell contact is required for HCV-mediated inhibition. While engaging NK CD81 with soluble α-CD81 abrogated NK inhibition, α-HCV E2 had no effect and IF microscopy revealed no cell surface HCV E2 on HCV-infected Huh-7.5. NK cell NKG2D and CD16 expression was unchanged over a 5h co-culture with HCV-infected Huh-7.5 cells, and surface levels of HLA-E did not increase on HCV-infected Huh-7.5 cells. In 5h co-culture with HCV-infected cells, NKp30 expression declined by 17%.

Conclusion(s): Extracellular HCV E2 does not interact with NK CD81 in vitro; however, the cell-to-cell contact-dependent inhibition of NK cells we observed occurs through an HCV E2-independent CD81-related pathway. Decreased NKp30 expression may directly contribute to inhibition of NK functions. If so, then elucidation of an up-regulated NKp30 ligand following HCV infection could identify new strategies to preserve NK functions in HCV-infected individuals.

Funding source (f): Supported by CIHR, NCRTP-Hep C and the Faculty of Medicine, Memorial University.

Poster number: 403

ALPHA DEFENSIN (HNP1) INTERFERES WITH THE HEPATITIS C VIRUS LIFECYCLE THROUGH IMPAIRED HCV PARTICLE RELEASE

Sonya MacParland [1], Alana Sherker [2], Vera Cherepanov [2], Haibo Zhang [3], Mario Ostrowski [1, 3], Jordan Feld [2, 4]
1. University of Toronto (Toronto, Canada); 2. McLauglin-Rotman Centre for Global Health, University Health Network (Toronto, Canada); 3. St. Michael's Hospital (Toronto, Canada); 4. Toronto Western Hospital (Toronto, Canada)

Background: Alpha-1 defensin, also known as human neutrophil peptide (HNP1), is an antimicrobial peptide produced by human neutrophils and peripheral blood mononuclear cells. HNP1 has been shown to have activity against RNA viruses, including HIV. Our previous data suggest that HNP1 can limit hepatitis C virus (HCV) entry and has a small effect on RNA replication but has no effect on HCV protein translation.

Purpose: In this study we will examine the ability of HNP1 to have an effect on HCV assembly and release. Through these investigations, we aim to fully elucidate how HNP1 affects the HCV life cycle.

Method: The effect of HNP1 on HCV particle release was assessed using single- cycle HCV production assay. Briefly, S29 cells (a gift from Dr. Rodney Russell at Memorial University of Newfoundland) were transfected with 10ug of RNA encoding HCV genotype 2a isolate JFH1. These cells are Huh-7 cells expressing minimal CD81 and are 1,000-fold less susceptible to HCV infection than Huh-7.5.1 cells and therefore virus spread after transfection is not expected. JFH1-transfected S29 cells were cultured in the presence or absence of 10 ug/mL of synthetic HNP1. As a positive control, JFH1-transfected S29 cells were treated for 6 hours with Brefeldin A to prevent excretion from the endoplasmic reticulum. 72 hours and 10 days later, intracellular HCV expression was examined by HCV core staining. Viability was assessed by 7'AAD staining. Intra- and extracellular HCV titers were determined from S29 cell lysates or supernatants, respectively, at 72 hours and 10 days posttransfection by qPCR. Both intracellular HCV RNA and supernatant will be used to infect naïve CD81-positive cells to assess if assembly of infectious viral particles is occurring properly. The ability of intrahepatic lymphocytes to produce HNP1 in response to various stimuli was also examined.

Result(s): Synthetic HNP1 treatment prior to transfection and throughout the culture period led to an accumulation of intracellular HCV core protein when compared to untreated HCV-transfected S29 cells. Synthetic HNP1 treatment also resulted in decreased cell apoptosis in relation to untreated controls. Our data suggest that HNP1 may interfere with the HCV lifecycle in multiple ways including impairment of HCV particle release. HNP1 is known to affect lipid homeostasis in the cell, which may be the common link explaining its effects on entry, replication and HCV assembly.

Conclusion(s): HNP1 may serve as a novel host-derived antiviral agent and may represent an effective tool to prevent infection in HCV-exposed individuals and after liver transplantation.

Funding source (f): National CIHR Training Program in Hepatitis C; CASL/CIHR Postdoctoral Fellowship

Poster number: 404

HCV LOAD AND EXPRESSION OF SELECTED CELLULAR GENES IN CIRCULATING LYMPHOID CELLS DIFFERENTIATE NONRESPONDERS FROM RESPONDERS TO PEG-IFN-RIBAVIRIN TREATMENT

Thomas Michalak [1], Tram NQ Pham [1], Dolly MH Lin [1], Patricia M. Mulrooney-Cousins [1], Norma D Churchill [1], Arleta Kowala-Piaskowska [2], Iwona Mozer-Lisewska [2], Krzysztof Simon [3], Dawn King [4], S. Bharati Reddy [4]
1. Molecular Virology and Hepatology Research Group, Faculty of Medicine, Memorial University (St. John's, Canada); 2. Department of Infectious Diseases, University of Medical Sciences (Poznan, Poland); 3. Department of Infectious Diseases, Hepatology and Acquired Immune Deficiencies, Medical University of Wroclaw (Wroclaw, Poland); 4. Hepatology Clinic, Eastern Health (St. John's, Canada)

Background: Some cellular genes have been implicated as correlates of successful or failed IFNα-Ribavirin (IFN/RBV) therapy against chronic hepatitis C (CHC). Such data were mostly derived from investigations of liver biopsy or plasma samples.

Purpose: To determine if expression of host genes thought to be relevant to HCV replication in the liver would be correlated with HCV infection status in PBMC and with patient responsiveness to IFN/RBV treatment.

Methods: PBMC from patients who responded (n=35) or not (n=41) to IFN/RBV therapy and from healthy controls (n=15) were evaluated for HCV RNA load and expressions of IFN (IFN-α, -β, -γ and -λ) and TLR (TRL-2, -3, -4, -5 and -7) gene families, IFN-inducible ISG15 and OAS, and IL-8, IL-10 and USP18 by real-time RT-PCR assays.

Results: Compared to IFN/RBV responders, nonresponders expressed significantly elevated levels of IL-8, ISG15, OAS, TLR-4, -5 and -7 (P from 0.040 to 0.0015) in PBMC before treatment, with IL-8, ISG15 and OAS remaining higher in the nonresponders post-therapy. Nonresponders with similar post-treatment follow-up as responders had 6-20-fold greater levels of IL-8, ISG15 and OAS post-treatment. In contrast, baseline levels of IFN-λ and TLR-3 were markedly higher (P =0.04) in responders, but such elevation was negated post-treatment. No notable differences in expression were observed for other genes, except IFN-α and IFN-γ whose expressions were higher (P=0.044) in responders after treatment. Pre-treatment HCV RNA loads in PBMC of nonresponders were significantly greater (P=0.016) than those of responders.

Conclusions: Elevated baseline HCV loads and levels of IL-8, ISG15 and OAS in PBMC of nonresponders compared to responders correlated with IFN/RBV therapy failure. The finding that post-treatment levels of IFN-α and IFN-γ were lower in nonresponders than in responders could be interpreted as a reaffirmation of a pivotal role of these cytokines in eliminating HCV from the lymphoid cell compartment. The results warrant further investigations on the utilization of PBMC for predicting success or failure of IFN-based therapies.

Funding sources: CIHR operating grant MOP-77544.

Poster number: 405

INVESTIGATING THE MODULATION OF FATTY ACID SYNTHASE (FASN) DURING INFECTION WITH HEPATITIS C VIRUS

Neda Nasheri [1]
1. University of Ottawa (Ottawa, Canada)

Background: Hepatitis C virus (HCV) induces lipid biogenesis and accumulation of lipid droplets (LDs) to facilitate its viral life cycle. Fatty acid synthase (FASN) is a multi-domain enzyme that plays a key role in the biosynthesis of fatty acids. Herein, we aimed to investigate the expression and activity of FASN during the HCV replication, and also in the presence of its individual proteins. We also examined the subcellular localization of FASN during HCV replication.

Method: We applied activity-based protein profiling, using the Orlisat-based probe, to investigate the alteration in the activity of FASN during HCV replication. Western blot analyses were performed to study the expression of FASN, and confocal microscopy was employed to examine the localization of FASN.

Result(s): • The activity and expression of FASN is increased significantly during HCV replication.
• Over-expression of Core and NS4B leads to an increase in the activity and expression of FASN.
• The increase in the expression and activity of FASN reflects in cellular triglyceride levels.
• The localization of FASN does not change during the HCV replication

Conclusion(s): The expression and activity of FASN increases significantly during HCV replication, and therefore FASN has the potential to become a prognostic and diagnostic marker for HCV-induced liver steatosis.

Funding source (f): CIHR, NCRTP-HepC

Poster number: 406

MIR-122 AND HEPATITIS C VIRUS: INVESTIGATING THE REQUIREMENTS OF AN UNUSUAL MICRORNA-TARGET RNA INTERACTION

Selena Sagan [1], Peter Sarnow [1]
1. Department of Microbiology & Immunology, Stanford University School of Medicine (Stanford, United States)

Background: 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' non-coding region (NCR) of the HCV genome. This is an unusual microRNA interaction in that it promotes HCV RNA accumulation. This interaction is important for maintaining HCV RNA levels in both HCV-infected cells and in the liver of infected chimpanzees, making miR-122 an attractive antiviral target. In fact, antisense locked nucleic acid inhibitors of miR-122 have currently gone through Phase II clinical trials for the treatment of HCV (Santaris Pharma a/s). Thus, it is imperative to gain information on the mechanism of this interaction and how the complex between miR-122 and the viral RNA differs from the microRNAs normal cellular targets. In addition, further elucidation of this complex will help identify novel antiviral targets.

Method: To investigate the requirements of the oligomeric complex of miR-122 molecules at the 5' end of the HCV genome, we have taken two strategies. In the first, we have prepared a novel tagging strategy for affinity purification of miR-122 complexes in HCV-infected and uninfected cells. In the second, we use a tethering approach to elucidate the requirements for protein complexes localized to the 5' end of the viral genome.

Result(s): Using this approach we will identify novel host and viral protein complexes associated with miR-122 at the 5' end of the viral genome and define the requirements for this unique microRNA:target RNA interaction.

Conclusion(s): Our recent findings that miR-122 interacts with the 5' terminus of the HCV genome and produces 3' overhanging extensions suggests novel hypotheses regarding its mechanism of action. Affinity purification and analyses of miR-122:HCV RNA complexes will further elucidate the host or viral factors associated with this complex and will identify novel antiviral targets to limit HCV accumulation.

Funding source (f): Amgen Fellow of the Life Sciences Research Foundation (LSRF)
National CIHR Research Training Program in Hepatitis C virus (NCRTP-HepC)

Poster number: 407

NEW SMALL MOLECULE AND NATURAL PRODUCT INHIBITORS OF HCV INFECTIVITY IDENTIFIED USING NOVEL ASSAYS FOR HCV ENTRY FUNCTIONS

Che Colpitts [1], Luis M Schang [1]
1. University of Alberta (Edmonton, Canada)

Background: We use chemical biology to study HCV replication, with a strong emphasis on entry, and to discover novel approaches to develop novel antivirals. We have identified and characterized the RAFIs (rigid amphipathic fusion inhibitors), nucleoside derivatives which inhibit HCV entry by targeting the lipids on the HCV envelope to inhibit the fusion of the virions to cell membranes. We have also characterized the antiviral mechanisms against HCV of a green tea polyphenol, epigallocatechin gallate (EGCG). EGCG inhibits the primary attachment of HCV virions to the glyco moieties in cellular glycosaminoglycans.

Purpose: We are applying the infectivity, attachment and fusion assays that we have developed, and developing new ones, to characterize the antiviral mechanisms against HCV of other small molecules and natural compounds.

Method: We have developed a series of assays for HCV entry. These assays use infectious HCV (strain JFH-1) and susceptible Huh7.5 cells, and recapitulate the actual requirements for HCV infectivity. The assays are based on R18-labeling of HCV virions and fluorescence analysis after attachment or fusion.

Result(s): Polyphenolic turmeric curcumin inhibits HCV infectivity (IC50, 8.5µM), whereas the non-planar related tetrahydrocurcumin (THC) did not. Curcumin has structural similarities with sterols, known modulators of membrane fluidity, and had been reported to promote the formation of negative curvature (which should promote fusion). We tested whether curcumin affected the fluidity of the HCV envelope. Using a 1,6-diphenyl-1,3,5-hexatriene (DPH) polarization assay that we developed, we found that curcumin decreases the fluidity of the HCV envelope as efficiently as cholesterol. Using the non-radioactive HCV attachment assays that we had developed, we found that curcumin inhibits attachment of HCV JFH-1 virions to Huh7.5 cells (IC50, 15µM), which is consistent with its effects on envelope fluidity. Also consistently with its effects on HCV envelope fluidity, 20 µM curcumin fully inhibited the fusion of HCV virions to Huh7.5 cells. Therefore, curcumin inhibition of HCV envelope fluidity, together with its resulting inhibition of binding affinity, overcomes its pro-fusogenic effects on the promotion of negative curvature resulting in the observed inhibition of HCV fusion and infectivity.

We are also using our assays to characterize the antiviral effects of sylimarin, a complex "Milk Thistle" extract, and its major components silibinin and silidianin, on HCV infectivity and replication. Silymarin and the two enantiomers of silibinin inhibited HCV infectivity (IC50, 10-12µg/ml). Silymarin main target was in the virions, as the IC50 was 6-fold lower when virions were directly exposed than when cells were treated after infection. Silymarin inhibited HCV attachment (IC50, 6µg/ml equivalent), whereas silibinin or its di-succinate ester did so at 12-fold higher concentrations and silidianin did not. Silymarin inhibited HCV fusion at 10µg/ml, whereas unconjugated silibinin did so at 100µg/ml, and its di-succinate ester or silidianin did not.

Conclusion(s): We have developed a series of biophysical and biochemical assays to analyze the effects of small molecules on early HCV entry and we are using these assays to elucidate novel antiviral mechanisms against HCV and to learn about its entry requirements.

Funding source (f): CIHR and BWF

Poster number: 408

HEPATITIS C VIRUS PROPAGATION IN HUMAN CD4+ AND CD8+ T LYMPHOCYTES

Georgia Skardasi [1], Thomas I. Michalak [1]
1. Molecular Virology and Hepatology Research Group, Faculty of Medicine, Memorial University (St. John's, Canada)

Background: Molecular and clinical evidence indicate that immune cells can support replication of HCV. Previously, an in vitro HCV replication system was established in which mitogen-induced PBMC-derived T cells served as targets for plasma occurring native HCV.

Purpose: To investigate the ability of native, patient-derived HCV to infect CD4+ and CD8+ T lymphocytes and to assess properties of the virions produced.

Method: Plasma from patients chronically infected with HCV, pretested for their ability to infect PBMC-derived T cells in vitro, served as inocula for infection of isolated normal human CD4+ and CD8+ T cells (>97% pure by flow cytometry).The target cells were pre-stimulated with phytohaemagglutinin (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 (MacParland et al., Hepatology 2008;49:1431; Sarhan et al., JVI 2012;86:3723) . HCV RNA positive (genomic) and negative (replicative) strands were detected by strand-specific RT-PCR followed by nucleic acid hybridization (RT-PCR/NAH) (Pham TNQ et al., JVI 2004;78:5867). Intracellular HCV NS5a and core proteins were identified by confocal microscopy. Released HCV RNA-reactive particles were examined by sucrose and iodixanol gradient ultracentrifugations. Clonal sequencing of the HCV 5'-UTR region served to compare the HCV virions harboured by inocula and released by infected cells.

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. Up to 1.2% cells were found NS5A protein positive. HCV RNA-reactive particles displaying a distinct sedimentation velocity and buoyant density than those in inocula occurred in culture supernatants from CD4+ and CD8+ T cells exposed to HCV. Clonal sequencing revealed different HCV variants in infected cells compared to infectious inocula.

Conclusion(s): Native, patient-derived HCV can infect and establish productive replication in normal human CD4+ and CD8+ T cells in vitro as evidenced by detection of HCV RNA replicative strand and intracellular expression of NS5a and core proteins. De novo HCV infection of the T cell subsets was confirmed by identification of HCV RNA-reactive particles in cell culture supernatants with distinct physical properties in comparison to those occurring in infectious plasma and by detection of unique HCV sequences in the in vitro infected cells.

Funding source (f): CIHR operating grant MOP-77544 and National CIHR Research Training Program in Hepatitis C.

Poster number: 409

HEPATITIS C VIRUS DURING PREGNANCY: MATERNAL HUMORAL IMMUNE RESPONSES AND VIRAL DYNAMICS OF INFECTING HCV POPULATIONS

Armelle Le Campion [1], Hugo Soudeyns [1], Ariane Larouche [1], Sébastien Fauteux-Daniel [1], Réginald Renoüs [1], Normand Lapointe [2], Marc Boucher [3], Deborah Money [4], Valérie Lamarre [5]
1. Unité d'immunopathologie virale, Centre de recherche du CHU Sainte-Justine (Montréal, Canada); 2. Centre maternel et infantile sur le SIDA, Centre de recherche du CHU Sainte-Justine (Montréal, Canada); 3. Service de gynéco-obstétrique, CHU Sainte-Justine (Montréal, Canada); 4. Women's Health Research Institute (Vancouver, Canada); 5. Service des maladies infectieuses, CHU Sainte-Justine (Montréal, Canada)

Background: The pathogenesis of hepatitis C during pregnancy remains poorly understood. Pregnancy is associated with a modulation in maternal immune responses which may influence the course of HCV disease. Markers of liver inflammation decline during the second and third trimester of pregnancy while HCV viral load increases. Furthermore, a significant decrease in HCV viral load is generally observed during the post partum period.

Purpose: To better understand how pregnancy-associated immune modulation influences the progression of HCV disease, viral dynamics of infecting HCV populations were studied. We hypothesized that variation in maternal HCV-specific humoral immune responses exerted differential selective pressures on HCV quasispecies as a function of the different stages of pregnancy.

Method: A high-resolution portrait of HCV quasispecies evolution based on hypervariable regions 1-3 (HVR1-3) of the E2 envelope protein is being carried out using ultra-deep sequencing (Roche 454 GS-FLX System and Titanium series reagents) in a group of pregnant women infected with HCV (n=15) or co-infected with HCV and HIV (n=30). Study subjects were participants to the CMIS mother-child cohort (CHU Sainte-Justine, Montreal). Serum samples were obtained at different times during gestation and during the post partum period. Immunoglobulin (Ig) isotype profiles (IgG1, IgG2, IgG3, IgG4, IgA, and IgM) were examined using multiplex assays. A longitudinal characterization of HCV E2-specific antibody responses was performed by neutralization assays based on HCV pseudoparticles (HCVpp) bearing autologous E2 envelope segments.

Result(s): Ig profiling only showed minor variations in the levels of each isotype during pregnancy and in the post partum period. In contrast, ultra-deep sequencing of HVR1-3 revealed the presence of highly complex and rapidly diversifying variant spectra, with dN/dS ratios consistent with significant selective pressure being exerted on the HCV envelope. Importantly, the emergence of novel major variants was observed during the post partum period concurrently with a decrease in HCV viral load. Based on these results, 10 different autologous HCVpp were generated, all of which were capable of infecting Huh7.5 cells.

Conclusion(s): This study provides a detailed parallel analysis of the dynamics of HCV quasispecies evolution and E2-specific humoral immune responses during pregnancy. Preliminary results suggest that reductions in HCV viral load observed in the post partum period are associated with quantitatively and qualitatively significant changes in the variant spectra, including emergence of novel major variants. Results from this study will better our understanding of the pathogenesis of hepatitis C in pregnancy and may lead to innovative approaches to treatment and to the prevention of mother to child HCV transmission.

Funding source (f): Canadian Institutes of Health Research (to HS, NL, VL, and MB).
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Poster number: 410

CHARACTERIZATION OF MIR-122-DEPENDENT AND -INDEPENDENT MECHANISMS OF HEPATITIS C VIRUS REPLICATION

Patricia Thibault [1, 2], Adam Huys [1, 2], Joyce Wilson [1, 2]
1. Department of Microbiology and Immunology (Saskatoon, Canada); 2. Vaccine and Infectious Disease Organization, University of Saskatchewan (Saskatoon, Canada)

Background: The liver-specific microRNA miR-122 has been implicated in various stages of the Hepatitis C Virus life cycle, from stabilizing and protecting the viral RNA from host cell nucleases, to enhancing translation of the viral RNA, to acting as the switch between translation and replication for viral RNAs. It is particularly challenging to dissect the role – or roles – of miR-122 in the viral life cycle, as previous studies lacked a critical control: HCV that replicates in the absence of miR-122. We previously identified miR-122-independent replication of sub-genomic (SGR) and full-genomic (FGR) di-cistronic HCV constructs, using the human hepatoma cell line Hep3B. We verified miR-122-independent replication by showing that it was not affected by a miR-122 antagonist (anti-122) in both Huh7.5 cells and Hep3B cells. We have further used the miR-122-independent system in proof-of-principle experiments to show that Argonaute-2 knockdown attenuates miR-122-dependent, but not miR-122-independent, HCV replication.

Purpose: The miR-122-independent system permits us to examine what role miR-122 plays in the Hepatitis C Virus life cycle by comparing effects of manipulations of miR-122-dependent and miR-122-independent replication. We can also use this system to identify the roles for miR-122 in different stages of the HCV life cycle.

Method: We employed the SGR construct in Hep3B and Huh7.5 cells, along with synthetic miR-122 agonists and antagonists, to establish roles for miR-122 at early and late stages of the HCV life cycle.

Result(s): SGR constructs were responsive to miR-122 agonists given either concomitantly with viral RNA, or supplied three days post-electroporation with viral RNA. Interestingly, miR-122-supported replication, although responsive to the anti-122 antagonist when given along with the viral RNA, was unaffected by anti-122 given three days after electroporation. Conversely, the mono-cistronic full-length J6/JFH-1 construct, which does not demonstrate miR-122-independent replication, was affected by both miR-122 agonist and antagonist given three days after electroporation.

Conclusion(s): As anticipated, we have used miR-122-independent HCV SGR replication to further characterize the role of miR-122 in the HCV life cycle. Preliminary data suggests that miR-122 plays multiple roles in the HCV life cycle, as addition of miR-122 can enhance both miR-122-dependent and -independent replication in di-cistronic and mono-cistronic constructs, but late addition of anti-122 only affects the mono-cistronic construct that is unable to carry out miR-122-independent replication. These data strongly support that there are at least two distinct functions for miR-122: one that supports the establishment of HCV replication and another that is required for ongoing replication of mono-cistronic full-length HCV genomes, but not di-cistronic HCV replicons. We will also use this system to verify other host proteins suspected to be involved in miR-122-mediated enhancement of HCV replication by comparison of their impact on miR-122-dependent vs. miR-122-independent replication.

Funding source (f): NCRTP-HepC, NSERC, and RAPID (SHRF)

Poster number: 411

IDENTIFICATION OF DOMINANT DRUG TARGETS IN HEPATITIS C VIRUS

Nicholas van Buuren [1], Jeannie Spagnolo [1], Ernesto Mendez [1], Karla Kirkegaard [1]
1. Stanford University (Palo Alto, United States)

Background: Over 170 million people are estimated to be infected with hepatitis C virus (HCV) worldwide. The current therapy is a combination of pegylated-interferon alpha and ribavirin, which is effective in roughly 50% of patients but coincides with high toxicity. The first FDA approved target-specific antivirals, Telaprevir and Boceprevir, target the NS3/4 protease and are prone to the development of drug resistance. Research in our laboratory has shown that not all direct-acting antivirals lead to the outgrowth of drug-resistant variants. Through targeting dominant drug targets, such as the poliovirus capsid, and potentially other oligomeric structures within infected cells, we have been able to repress the outgrowth of drug-resistant variants. This paradigm includes all situations where drug-bound viral proteins can function as dominant negatives to repress the outgrowth of all viruses within the quasispecies. We are interested in those drugs that generate recessive drug-resistant variants; that is, those that, because their targets are oligomeric, will suppress the outgrowth of drug-resistant variants. These studies have the potential to radically change HCV anti-viral design.

Purpose: To identify targets for direct-acting antivirals that repress the outgrowth of drug-resistant HCV variants.

Method: We have passaged the JFH1 strain of HCV in Huh7.5.1 cells at low MOI in the presence of suboptimal concentrations of a variety of direct-acting antivirals in order to select for drug resistant variants. The antivirals that we have synthesized are BILN-2061, which targets the NS3/4A protease, MK0608 and R1479, which target the NS5B polymerase and SR2486, which targets the NS5A phosphoprotein. The passaged virus was sequenced to identify coding mutations, and these were then cloned into plasmids encoding the HCV genome. Individual variants were then grown and screened for drug resistance.

Result(s): We have developed two HCV variants with resistance to the protease inhibitor BILN-2061. Both strains contain the previously described D168A mutation located near the active site of the HCV protease. Additionally, one strain carries a M581T mutation while the other carries an L386V mutation. Significantly, both D168A/L386V and D168A/M581T display strong resistance to 2uM BILN-2061 at 36 and 72hr post infection. Our next step is to perform co-infections to determine if either of these strains are recessive to wild type HCV in the presence of BILN-2061.
HCV was passaged in the presence of two polymerase inhibitors, MK0608 (Merk) and R1479 (Roche). Three individual mutations that potentially confer resistance to MK0608 were identified: A336P, S280T and D438G. Additionally, we identified a double mutation, F427L/T481A, that we suspect confers resistance to R1479. We are currently in the process of amplifying these HCV variants in order to screen them for drug resistance. HCV passaged in the presence of SR2486 is currently being sequenced to identify unique coding mutations. Drug resistant variants will then be used to perform co-infections with the aim of identifying novel dominant drug targets within HCV.

Conclusion(s): Our research aims to further characterize the virus-virus interactions that take place in the context of mixed infections and how these contribute to the outgrowth of HCV variants with the goal of decreasing drug resistance to direct acting antivirals.

Funding source (f): NIH, NIH Pioneers Award

Poster number: 412

MAPPING OF CROSS-NEUTRALIZING ANTIBODY EPITOPES TARGETED BY A RECOMBINANT HEPATITIS C VIRUS gpE1/gpE2 VACCINE CANDIDATE IN PHASE 1 CLINICAL TRIAL

Jason Wong [1], John Law [1], Rakesh Bhat [1], Aviad Levin [1], Sharon Frey [2], D. Lorne Tyrrell [1], Michael Houghton [1]
1. Li Ka Shing Institute of Virology, Department of Medical Microbiology and Immunology, University of Alberta (Edmonton, Canada); 2. St Louis University Medical School, Division of Infectious Diseases and Immunology (St. Louis, United States)

Background: There are ~160 million global carriers of the hepatitis C virus (HCV) with hundreds of thousands of new infections each year indicating an urgent need for a global vaccine. A vaccine derived from the two viral envelope glycoproteins gpE1/gpE2 was protective in chimpanzees, suggesting that a vaccine that generates cross-neutralizing antibodies could facilitate clearance of acute viremia, thus preventing viral persistence and associated disease. A recombinant gpE1/gpE2 vaccine candidate based on a single 1a isolate has been tested in a phase 1 clinical trial. Cross-neutralizing activity against all global clades of the virus has been demonstrated in vitro and this has prompted investigations into the responsible epitopes and mechanisms of action. Goats have been immunized with the clinical vaccine and the antibodies elicited are also being investigated similarly.

Purpose: To determine the epitopes targeted by vaccine-mediated cross-neutralizing antibodies.

Method: Several human and mouse monoclonal antibodies (mAbs) with cross-neutralizing activity and defined target epitopes have been reported in the literature. Such antibodies are being used in competition ELISAs using vaccinee antisera.
Peptide binding assays are being used to map the epitopes recognised by vaccinee antisera and rabbit antisera raised against reactive peptides are being assayed for cross-neutralizing activity,

Result(s): The use of overlapping peptide arrays to detect antibody-targeted epitopes has revealed several known as well as potentially novel cross-neutralizing epitopes within both gpE1 and gpE2. Currently, these are being confirmed using the above methods.

Conclusion(s): We have demonstrated cross-neutralizing activity in vitro in human volunteers immunized with the glycoproteins from a single 1a isolate. Determining the areas on the glycoproteins that are responsible for broad cross-neutralization of the many variable HCV clades remains an important goal in future vaccine design.

Funding source (f): National CIHR Research Training Program in Hepatitis C (Jason Wong); Canadian Excellence in Research Chair (Michael Houghton).

Poster number: 413

THE EFFECT OF PTEN ON HCV INFECTION

Qi Wu [1], Qiang Liu [1]
1. *VIDO-InterVac, University of Saskatchewan (Saskatoon, Canada)*

Background: Hepatitis C virus (HCV) infection causes serious global public health problems. The World Health Organization has established that there are more than 170 million chronic HCV patients worldwide. Hepatocellular carcinoma (HCC) is the most deadly clinical consequence of HCV infection. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a tumor suppressor which is frequently mutated or deleted in tumors including HCC. However, the role of PTEN in HCV replication and pathogenesis is not well characterized. PTEN protein contains an N-terminal PIP2 (phosphatidylinositol-4,5-bisphosphate)-binding motif, a phosphatase domain, a C2 domain, a C-terminal tail containing two PEST (proline, glutamic acid, serine, threonine) sequences and a PDZ (PSD-95/DLG/ZO-1)-binding interaction motif at the end. Two naturally occurring mutations on the phosphatase domain disrupt PTEN's phosphatase activity: C124S mutation, which abrogates both lipid and protein phosphatase activity, and G129E mutation, which abrogates lipid phosphatase only.

Purpose: To determine the effect of PTEN on HCV replication and the underlying molecular mechanisms.

Method: We will characterize HCV replication after PTEN overexpression or knocking down PTEN expression. We will determine whether PTEN interacts with HCV viral proteins as a mechanism for its effect on HCV replication.

Result(s): In HCV JFH-1 genomic replicon cells, we showed that knocking down PTEN by shRNA significantly enhanced HCV replication. Consistently, PTEN overexpression significantly inhibited HCV replication. We further showed that the phosphatase domain was involved in HCV replication inhibition. Interestingly, PTEN with the lipid phosphatase defective mutation (G129E) could no longer inhibit HCV replication. In GST pull-down assays, we showed that HCV core protein interacted with PTEN.

Conclusion(s): The lipid phosphatase activity of PTEN is required for inhibiting HCV replication. HCV core interacts with PTEN, which may contribute to PTEN's effect on HCV replication. Our study may help justify further development of PTEN as a new drug target for HCV therapy.

Funding source (f): NCRTP, SHRF, CIHR

Participants list – Liste des participants

Abdel Hakeem	Mohamed	Université de Montréal (CRCHUM)	m.salah.eldin.abdel.hakeem@umontreal.ca
Anderson	Scott	CATIE	sanderson@catie.ca
Anonychuk	Andrea	Abbott Diagnostics	andrea.anonychuk@abbott.com
Artenie	Andreea Adelina	McGill University	adelina.artenie@mail.mcgill.ca
Bacon	Patricia	Blood Ties Four Directions Centre	executivedirector@klondiker.com
Baril	Jean	Hoffmann-La Roche	jean.baril@roche.com
Barker	Lara Lise	CATIE	lbarker@catie.ca
Bialystok	Frank	University of Toronto	
Bilodeau	Marc	NCRTP-HepC	marc.bilodeau@umontreal.ca
Brissette	Suzanne	CHUM	suzanne.brissette@umontreal.ca
Bruneau	Julie	CRCHUM, Université de Montréal	julie.bruneau@umontreal.ca
Buller-Taylor	Terri	BC Centre for Disease Control	terri.buller-taylor@bccdc.ca
Butt	Gail	BCCDC	gail.butt@bccdc.ca
Byrne	Marianne	Kirby Institute, University of New South Wales	mbyrne@kirby.unsw.edu.au
Choucha	Norma	NCRTP-HepC	ncrtp.hepc@gmail.com
Daniel	Judy	Janssen Inc.	jdaniel7@its.jnj.com
Day	Irene	BC Centre for Excellence in HIV/AIDS	iday@cfenet.ubc.ca
Depla	Marion	CR-CHUM	m.depla@hotmail.fr
Desjardins	Elyse	Janssen Inc	edesjard@its.jnj.com
Dodd	Zoe	Toronto Hepatitis C Community Program	zdodd@srchc.com
Dore	Gregory	The Kirby Institute-University of New South Wales	gdore@kirby.unsw.edu.au
Doyle	Mary-Anne	University of Ottawa	madoyle@TOH.on.ca
Fagan	Gary	Canadian Liver Foundation	gfagan@liver.ca
Fahmy	Ahmed	INRS-Institut Armand Frappier	ahmed.fahmy@iaf.inrs.ca
Falconer	Frances M V	Hepatitis C Support Nursing	hepcsupport@shaw.ca
Fauteux-Daniel	Sebastien	CHU Sainte-Justine Research Center	sebastien.fauteux-daniel@umontreal.ca
Feld	Jordan	TWH Liver Center - University Health Network	Jordan.Feld@uhn.ca
Fischer	Benedikt	Faculty of Health Sciences/SFU	bfischer@sfu.ca
Gendron	Pierre	Abbvie	pierre.gendron@abbvie.com
Götte	Matthias	McGill University	matthias.gotte@mcgill.ca
Grebely	Jason	University of New South Wales	jgrebely@kirby.unsw.edu.au
Green	Colin	Hepatitis Outreach Society of Nova Scotia	director@hepns.ca
Gulzar	Naveed	Simon Fraser University	ngulzar@sfu.ca
Hew	Huong	Janssen Inc.	hhew@its.jnj.com
Hoffman	Brett	University of Saskatchewan	b.hoffman@usask.ca
Holder	Kayla	Memorial University of Newfoundland	lkaylaaholder@gmail.com
Houghton	Michael	University of Alberta	michael.houghton@ualberta.ca
Hyatt	Amy	Janssen Inc	ahyatt@its.jnj.com
Jacka	Brendan	Kirby Institute	bjacka@kirby.unsw.edu.au
Johnston	Paul	Sherbourne Health Centre	pjohnston@sherbourne.on.ca
Khan	Mark	Gilead Sciences Canada Inc.	mark.khan@gilead.com
Khan	Mohammad Adil	Boehringer Ingelheim (Canada) Ltd	mohammad.khan@boehringer-ingelheim.com
Khan	Zaman	Abbott Laboratories, Diagnostic Division	zaman.khan@abbott.com
Krahn	Murray	THETA Collaborative, University of Toronto	murray.krahn@theta.utoronto.ca
Krajden	Mel	University of British Columbia	Mel.Krajden@bccdc.ca
Kulkarni	Anupriya	McGill University	anupriya.kulkarni@mail.mcgill.ca
Laird	Douglas	University of Victoria	bioxyzen@yahoo.ca
Laporte	Alexandre	CAPAHC and canadian coalition	alex.laporte@hepatitesressources.com
Lico	Steve	Janssen Inc.	slico@its.jnj.com

Liu	Qiang	University of Saskatchewan	qiang.liu@usask.ca
Machala	Anna	Janssen Inc.	amachala@its.jnj.com
MacParland	Sonya	University of Toronto	sonyamacparland@gmail.com
Marks	Pip	Kirby Institute	pmarks@kirby.unsw.edu.au
McGuinness	Liza	BC Centre for Disease Control	liza.mcguinness@bccdc.ca
McPhail	Hayley	PerCuro Clinical Research	hayley@percuro.net
Mersilian	Laurence	CAPAHC	direction@capahc.com
Michalak	Thomas	Faculty of Medicine, Memorial University	timich@mun.ca
Misurski	Derek	AbbVie	derek.misurski@abbvie.com
Mitchell	Sandi	BCCDC/University of Sydney	sandi.mitchell@bccdc.ca
Miyai	Wes	Merck Canada	wes.miyai@merck.com
Moqueet	Nasheed	McGill University	nsmoqueet@gmail.com
Morin	Sebastien	Merck Canada	sebastien.morin@merck.com
Mugford	Gerry	Memorial University of Newfoundland	gmugford@mun.ca
Nasheri	Neda	University of Ottawa	nasherin@nrc.ca
Njihia	Jane	Public Health Agency of Canada	jane.njihia@phac-aspc.gc.ca
Ollner	Annika	PASAN	annika@pasan.org
Olmstead	Andrea	BCCDC	andrea.olmstead@bccdc.ca
Peterson	Laila	PerCuro Clinical Reseach	laila@percuro.net
Pezacki	John	University of Ottawa	John.Pezacki@nrc-cnrc.gc.ca
Potkonjak	Billie	Canadian Liver Foundation	bpotkonjak@liver.ca
Rajotte	Daniel	Boehringer Ingelheim (Canada) Ltd	daniel.rajotte@boehringer-ingelheim.com
Raven	Jennifer	CIHR Institute of Infection and Immunity	jennifer.raven@crchuq.ulaval.ca
Reitz	Cheryl	HepCBC Hepatitis C Education & Prevention Society	cherylreitz@gmail.com
Rhodes	Tim	London School of Hygiene and Tropical Medicine	Tim.Rhodes@lshtm.ac.uk
Rice	Jeff	CATIE	jrice@catie.ca
Richardson	Christopher	Dalhousie University	chris.richardson@dal.ca
Roberts	Eve	University of Toronto	
Russell	Rodney	Memorial University/Health Sciences Centre	rodney.russell@med.mun.ca
Sagan	Selena	Stanford University	smsagan@stanford.edu
Schang	Luis	University of Alberta	luis.schang@ualberta.ca
Schmitz	Deb	Canadian Coaltion of Organizations Responding to Hepatitis B and C	deb@pacifichepc.org
Sebastiani	Giada	Mcgill University	giada.sebastiani@mcgill.ca
Seto	Karen	Canadian Liver Foundation	kseto@liver.ca
Sheristobitoff	Alex	Pacific Hepatitis C Network	alsher@shaw.ca
Sherman	Morris	University of Toronto	morris.sherman@uhn.on.ca
Singaravelu	Ragunath	National Research Council Canada	Ragunath.Singaravelu@nrc.ca
Skardasi	Georgia	Memorial University of Newfoundland	gs0667@mun.ca
Smith	Bryce	Centers for Disease Control and Prevention	blil@cdc.gov
Sockalingam	Sanjeev	University Health Network, University of Toronto	sanjeev.sockalingam@uhn.ca
Soudeyns	Hugo	Centre de recherche du CHU Sainte-Justine	hugo.soudeyns@recherche-ste-justine.qc.ca
Sung	Danny	Coalition of Organizations Responding to Hepatitis B and C	msa141@sfu.ca
Sutton	Paul	CTAC/Canadian Coalition of Organizations Responding to Hepatitis B and C	paul@ctac.ca
Szmukier	Zuzanna	Boehringer Ingelheim (Canada) Ltd	zuzanna.szmukier@boehringer-ingelheim.com
Tell	Lorraine	Merck Canada	lorraine.tell@merck.com
Tellier	Raymond	University of Calgary	Raymond.Tellier@albertahealthservices.ca
Thibault	Patricia	University of Saskatchewan	patricia.thibault@usask.ca
Trépanier	Janie	Merck Canada	janie.trepanier@merck.com
Trubnikov	Maxim	Public Health Agency of Canada	maxim.trubnikov@phac-aspc.gc.ca
Tuscano	Hywel	CATIE	htuscano@catie.ca

Tyrrell	D. Lorne	University of Alberta - Li Ka Shing Institute of Virology	lorne.tyrrell@ualberta.ca
Uruqhart	Stephen	Abbott	stephen.urquhart@abbott.com
Usaty	Chris	Roche	chris.usaty@roche.com
Van Buuren	Nicholas	Stanford University	njv@stanford.edu
Wakita	Takaji	National Institute of Infectious Diseases	wakita@nih.go.jp
Wang	Tianyi	University of Pittsburgh	tywang@pitt.edu
Wansuanganyi Jean-Marie	Bamvita	CR-CHUM	wansuanganbamvita@sympatico.ca
Wilson	Joyce	University of Saskatchewan	joyce.wilson@usask.ca
Wong	Jason	Houghton Lab, Li Ka Shing Institute of Virology, University of Alberta	jasonale@ualberta.ca
Wong	Tom	Public Health Agency of Canada	Tom_Wong@phac-aspc.gc.ca
Wu	Qi	VIDO-InterVac, University of Saskatchewan	qiw186@mail.usask.ca
Yu	Amanda	BC Centre for Disease Control	amanda.yu@bccdc.ca

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