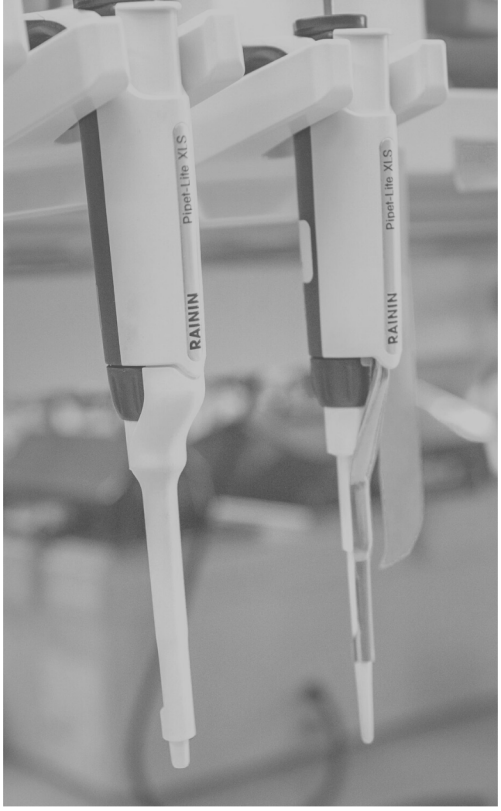
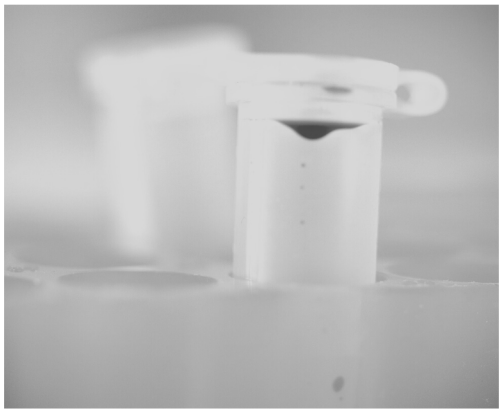




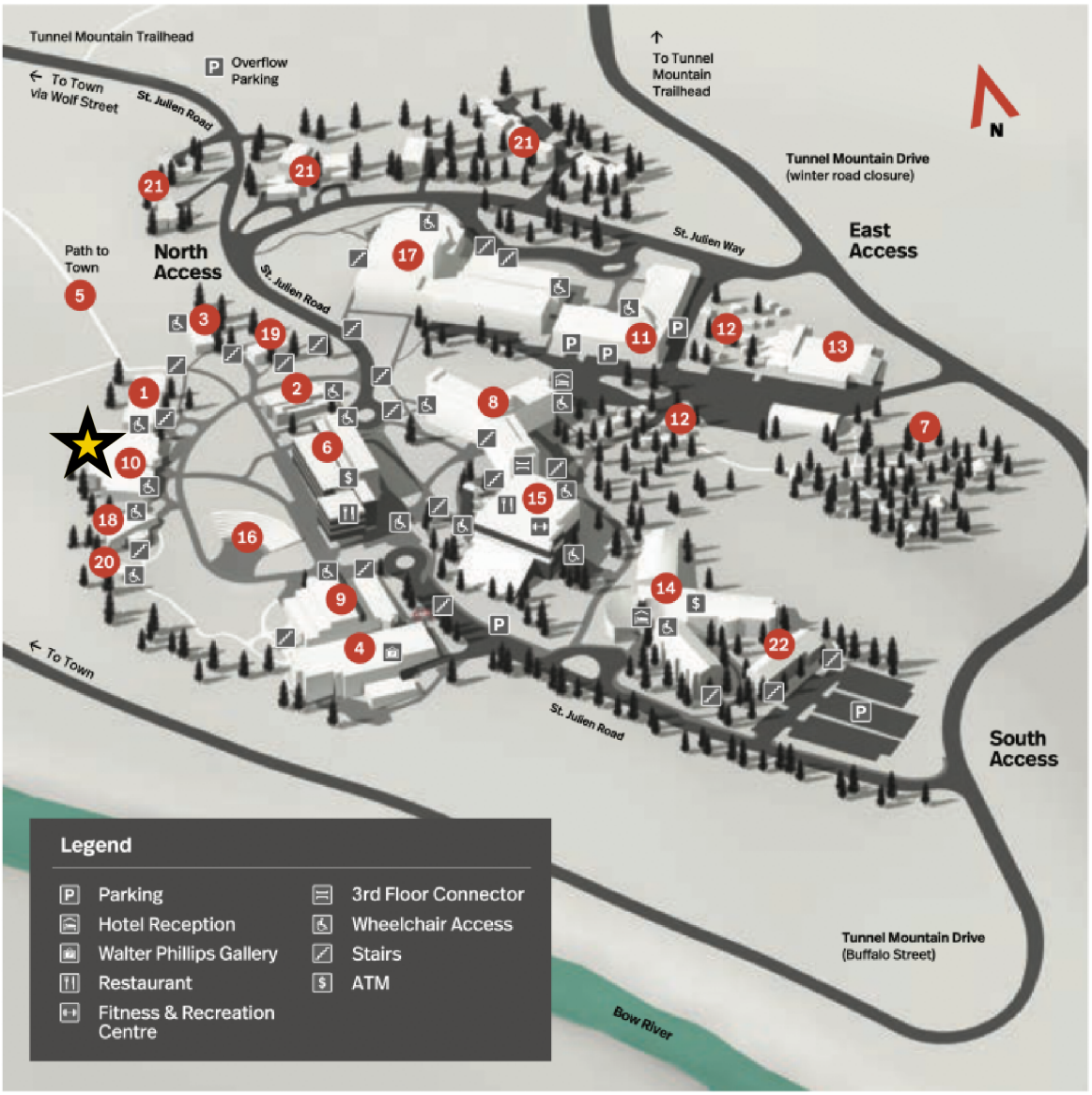
SPP-ARC IGNITES INNOVATION

Annual Retreat

November 16-18 | 2023

Banff Centre for Arts and Creativity

Campus Map



1. **Corbett Hall**
2. **Donald Cameron Centre**
   Administration Offices
3. **Farrally Hall**
4. **Glyde Hall**
   Walter Phillips Gallery
5. **Ken Madsen Path to Town**
6. **Kinnear Centre for Creativity & Innovation**
   Maclab Bistro
   Meeting Rooms & Banquets
   Paul D. Fleck Library & Archives
7. **Leighton Artists Studios**
8. **Lloyd Hall**
   Hotel Reception
9. **Jeanne & Peter Lougheed Building**
10. **Max Bell Building**
11. **Music Building**
    Bentley Chamber Music Studio
    Rolston Recital Hall
12. **Music Huts**
13. **Physical Facilities Building**
    Print Shop
    Shipping & Receiving
14. **Professional Development Centre**
    Hotel Reception
15. **Sally Borden Building**
    Fitness & Recreation Centre
    Participant Resources
    Three Ravens Restaurant
    Vistas Dining Room
16. **Shaw Amphitheatre**
17. **Theatre Complex**
    Box Office
    Jenny Belzberg Theatre
    Laszlo Funtek Teaching Wing
    Margaret Greenham Theatre
    The Club
18. **TransCanada PipeLines Pavilion**
    Banff International Research Station
19. **Vinci Hall**
20. **Yurt**
21. **Staff Housing**
22. **Staff Housing**
    Becker Hall

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Welcome from Co-Directors

Dear Participants,

Thank you for attending the first annual SPP-ARC retreat. The consortium is funded by a $55.1 million grant from the Government of Alberta, Ministry of Jobs, Economy and Trade. Our mission is to develop capacity and capabilities that support innovative R&D efforts for better protection against emerging pathogens. The discovery, development, and assessment of vaccines and small molecule antiviral drugs is an important cornerstone in this regard. Hence, the focus of the inaugural meeting will be on specific research & training activities that involve the entire team.

Funding has been secured for a period of five years from 2022 to 2027. In the first year, we established a governance structure with a steering committee, a scientific advisory board, an administrative core and several research committees. We developed funding mechanisms for research, infrastructure and training projects that are aligned with our mandate. The foundational grant has been successfully leveraged for the recruitment of highly qualified personnel and to obtain substantial additional funding at the national and international levels. Our vision is to become a global leader in the field of pandemic preparedness. To make significant contributions to the development of effective medical countermeasures is a specific objective.

SPP-ARC investigators cover a broad range of complementary expertise in the areas of virology, immunology, biochemistry, medicinal chemistry and structural biology. The retreat provides opportunities to initiate team activities and create synergy among the various individual research labs.

We thank you for your attendance and look forward to inspiring discussions throughout this meeting.

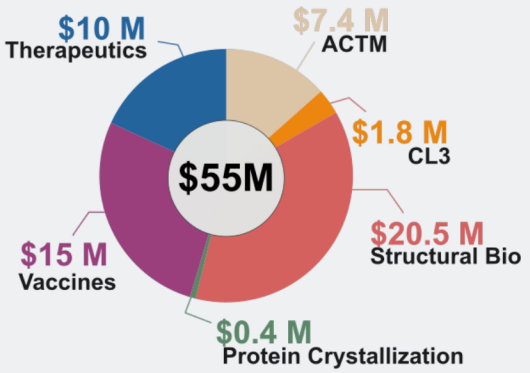
Sincerely,
SPP-ARC Co-Directors

Dr. Matthias Götte

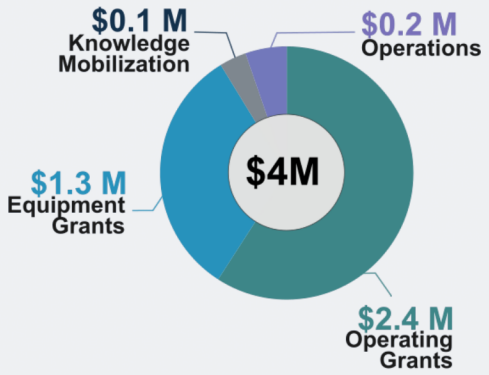
Dr. D. Lorne Tyrrell

SPP-ARC at a Glance

BUDGET



RESEARCH EXPENSES



TOTAL EXPENSES TO DATE

22 Researchers funded

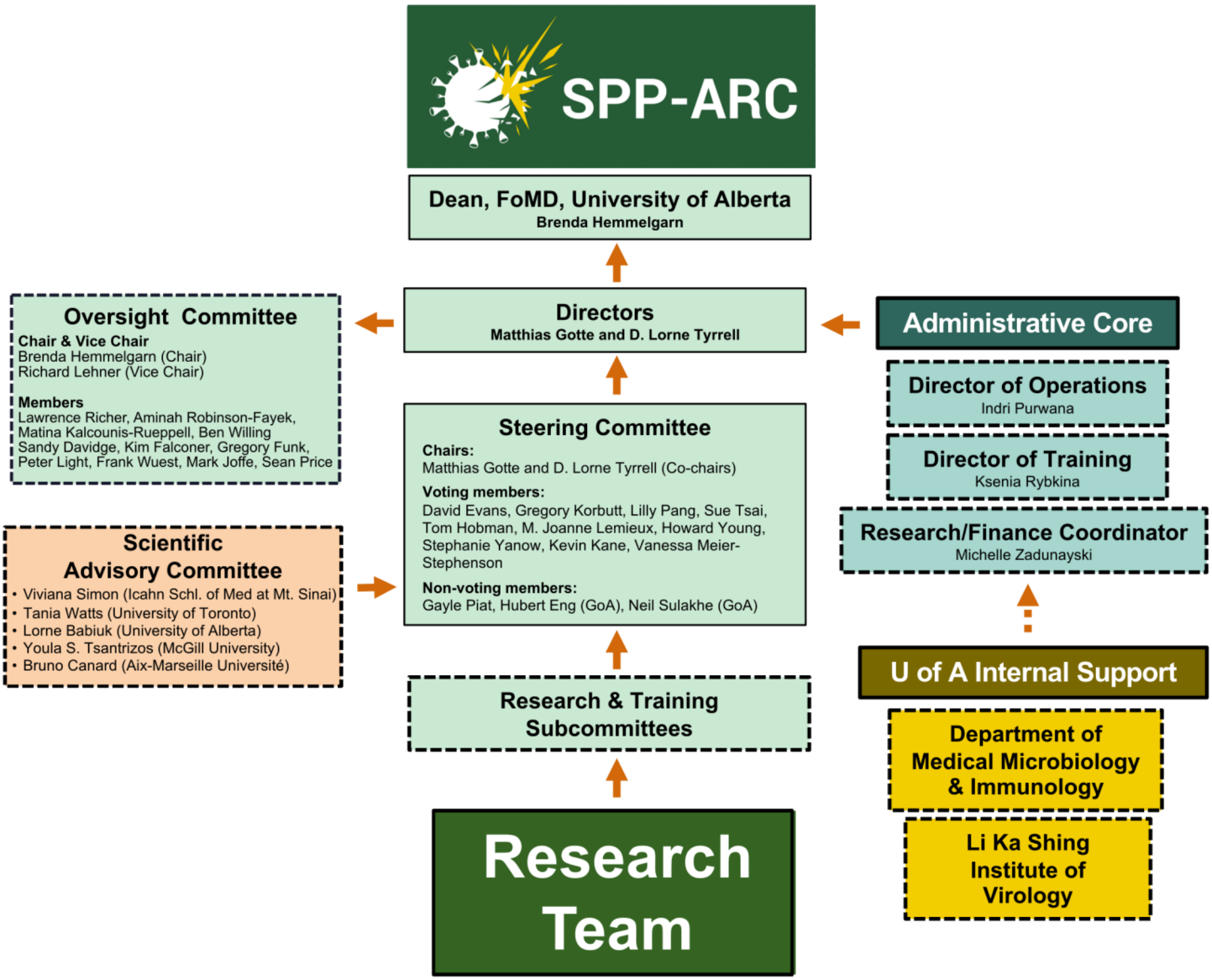
17 Equipment funded

19 Projects funded

7 Workshops Seminars

Gene Synthesizer
2MP Mass Spectrometer
ELISPOT Analyzer
Electroporator
Nanoparticle Analyzer
Light/IF Microscope
Cell Homogenizer
Spectrophotometer
Purification System
Microfluidic System

SPP-ARC Governance



Retreat Program

Thursday, November 16th, 2023

12:00 PM	Lunch	VISTAS DINING ROOM SALLY BORDEN BUILDING
1:30 PM	Poster Presentation & Competition	MAX BELL BUILDING 251/253
2:30 PM	Opening remarks from SPP-ARC Co-Directors • **Matthias Götte** • **Lorne Tyrrell**	MAX BELL BUILDING - 252
3:00 PM	SPP-ARC Expertise Cores Presentations • **Maya Shmulevitz/Troy Baldwin:** Vaccine & Immune Responses • **Matthias Götte**: Small Molecule Discovery • **David Marchant:** Molecular Virology • **Lorne Tyrrell/ Vanessa Meier-Stephenson:** Clinical Specimens	MAX BELL BUILDING - 252
4:10 PM	Break	

4:30 PM	Scientific Advisory Board Presentations
	• **Tania Watts (University of Toronto)**
	  ◦ Lessons learned from studying T cell responses to SARS-CoV-2 infection and vaccination
	• **Youla Tsantrizos (McGill University)**
	  ◦ COVID-19: What did we learn about the discovery of small molecule antivirals? … or didn't we?
	• **Lorne Babiuk**
	  ◦ Linking science to innovation and product development
		MAX BELL BUILDING - 252

6:00 PM	Keynote Presentation • **Andres Finzi (University of Montréal)** ◦ Humoural responses against the SARS-CoV-2 Spike: beyond viral neutralization	MAX BELL BUILDING - 252
7:00 PM	Dinner & Networking *cash bar available!	KINNEAR CENTRE 305

Retreat Program

Friday, November 17th, 2023

7:00 AM	Breakfast	VISTAS DINING ROOM SALLY BORDEN BUILDING

8:30 AM	Cryo-Electron Microscopy (Cryo-EM) and its applications
	• **Kalyan Das (Rega Institute, KU Leuven, Belgium)**
	  ◦ Cryo-EM structures illustrate step-by-step yeast mitochondrial transcription initiation
	• **Elizabeth Campbell (Rockefeller University, New York, NY)**
	  ◦ Structural studies of SAR-CoV-2 replicase complexes
		MAX BELL BUILDING 252

10:00 AM	Break	
10:30 AM	Facilities Presentations • **Howard Young:** Structural Biology Facility • **Gayle Piat:** Alberta Cell Therapy Manufacturing Facility • **David Evans:** Containment Level 3 Facility	MAX BELL BUILDING - 252
11:15 AM	Training Presentation • **Ksenia Rybkina:** Training in SPP-ARC - A Vision	MAX BELL BUILDING - 252
11:50 AM	Lunch	VISTAS DINING ROOM SALLY BORDEN BUILDING
1:30 PM	Breakout Rooms: 1. Trainees discussion (MB252) • **Meet & Greet Bruno Marchand (Gilead Sciences, CA)** ◦ Life outside academia • Feedback on SPP-ARC Training Program/Support 2. Small Molecule & Molecular Virology Core discussion (MB251) 3. Vaccines and Immune Response Core discussion (MB253) 4. Clinical Specimen Core discussion (MB155) 5. Cryo-EM discussion (MB156) • **Seth Darst (Rockefeller University, New York, NY)** • **Kalyan Das (Rega Insitute, KU Leuven, Belgium)** • Cryo-EM facility disucssion	MAX BELL BUILDING
3:30 PM	Break	

Retreat Program

Friday, November 17th, 2023 (continued)

4:00 PM	Vision Presentations • **Maya Shmulevitz/Troy Baldwin:** Vaccine & Immune Responses • **Matthias Götte:** Small Molecule Discovery • **David Marchant:** Molecular Virology • **Lorne Tyrrell/Vanessa Meier-Stephenson:** Clinical Specimens	MAX BELL BUILDING - 252
4:30 PM	Keynote Presentation • **Aled Edwards (University of Toronto)** *remote* and **Ryan Merkley (Viral Medicine Initiative (VIMI))** ◦ Pandemic drug discovery – prioritizing global and equitable access	MAX BELL BUILDING - 252
5:40 PM	Closing Remarks	MAX BELL BUILDING - 252
6:00 PM	Dinner & Networking *note: Buffet will close at 7:30 PM	VISTAS DINING ROOM SALLY BORDEN BUILDING

Saturday, November 18th, 2023

7:00 AM	Breakfast (until 9:30 if not leaving by bus)	VISTAS DINING ROOM SALLY BORDEN BUILDING
8:00 AM	Bus departure to UofA	PROFESSIONAL DEVELOPMENT CENTRE

Abstracts

INVITED SPEAKERS

HUMORAL RESPONSES AGAINST THE SARS-COV-2 SPIKE: BEYOND VIRAL NEUTRALIZATION

Andres Finzi University of Montréal, Québec, Canada

From the bench to the bedside: I will present some insights on how performing basic research on SARS-CoV-2 Spike conformation generated useful information on seroprevalence, convalescent plasma transfer, infection/vaccine-elicited responses, variants of concern and led to the identification and characterization of neutralizing antibodies with potent Fc-effector functions that completed a Phase II clinical trial.

STRUCTURE-BASED DRUG DESIGN USING CRYO-ELECTRON MICROSCOPY

Kalyan Das Rega Institute, KU Leuven, Belgium

The life cycle of many viruses depends on the expression of two key enzymes, a protease enzyme, which is responsible for the proteolytic processing of the viral polyprotein (after this polyprotein is translated in the host cell), and a polymerase enzyme, which is responsible for replicating the viral genome. Synthetic nucleosides are an important class of therapeutic drugs that target the active site of the viral polymerase enzymes. In contracts, protease inhibitors are designed to block the proteolytic cleavage of the viral polyprotein. Mechanistically, protease enzymes are broadly classified as (a) those using the nucleophilic side chain of an amino acid residue in order to initiate amide bond hydrolysis, such as serine proteases (e.g. HCV NS3/4A protease) or cysteine proteases (e.g. SARS CoV-2 3CLpro), and (b) those using an activated water molecule as the nucleophile that attacks the scissile amide bond, such as aspartate proteases (e.g. the HIV protease) and zinc metalloproteases [e.g. the host angiotensin-converting enzyme 2 (ACE2), which has been implicated in SARS CoV-2 infectivity and the severity of this disease]. Some lessons re-learned about the discovery of antivirals and successfully re-applied in the design of human therapeutics for COVID-19 will be described.

COVID-19: WHAT DID WE LEARN ABOUT THE DISCOVERY OF SMALL-MOLECULE ANTIVIRALS? ...OR DIDN'T WE

Youla S. Tsantrizos McGill University, Québec, Canada

The life cycle of many viruses depends on the expression of two key enzymes, a protease enzyme, which is responsible for the proteolytic processing of the viral polyprotein (after this polyprotein is translated in the host cell), and a polymerase enzyme, which is responsible for replicating the viral genome. Synthetic nucleosides are an important class of therapeutic drugs that target the active site of the viral polymerase enzymes. In contracts, protease inhibitors are designed to block the proteolytic cleavage of the viral polyprotein. Mechanistically, protease enzymes are broadly classified as (a) those using the nucleophilic side chain of an amino acid residue in order to initiate amide bond hydrolysis, such as serine proteases (e.g. HCV NS3/4A protease) or cysteine proteases (e.g. SARS CoV-2 3CLpro), and (b) those using an activated water molecule as the nucleophile that attacks the scissile amide bond, such as aspartate proteases (e.g. the HIV protease) and zinc metalloproteases [e.g. the host angiotensin-converting enzyme 2 (ACE2), which has been implicated in SARS CoV-2 infectivity and the severity of this disease]. Some lessons re-learned about the discovery of antivirals and successfully re-applied in the design of human therapeutics for COVID-19 will be described.

LINKING SCIENCE TO INNOVATION AND PRODUCT DEVELOPMENT

Lorne Babiuk

Development of biologicals requires a continuum of activities and expertise starting at fundamental research – mostly carried out in an academic environment. This phase generally moves ideas through the first three Technology Readiness Levels (TRL 1-3). However even this phase requires team work because of the varied expertise required. This will be demonstrated using infectious diseases and vaccines as an example. The next Technology Readiness Levels (TRL4-6) can involve academics but also require researchers in companies to test the ideas in a simulated world. These earlier phases are generally less costly that the final phase TRL 7-9 where the idea is tested in the real world and require extensive understanding of the regulatory process and quality control to ensure reproducibility as well as ensuring that the product does what it is designed to do. This final phase is not only the most expensive but also where the costliest failures occur. As a result, very few ideas actually get across the various valleys and cliffs that are traversed during the lengthy process. During the presentation the various phases of vaccine development will be described as will the importance that academics recognize the importance of team work and be prepared to hand of the project to others with different expertise for ultimate success.

LESSONS LEARNED FROM STUDYING T CELL RESPONSES TO SARS-COV-2 INFECTION OR VACCINATION

Tania Watts University of Toronto, Ontario, Canada

T cells are important in elimination of infected cells and in help for B cell responses. While viruses frequently mutate to evade neutralization, T cell epitopes tend to be distributed throughout the viral genome, making it difficult for viruses to evade T cells. Therefore, measuring T cell responses can provide important additional information over measuring antibodies alone. During the COVID-19 pandemic we studied T cell responses to SARS-CoV-2 in convalescent patients. Later, we investigated how immunosuppressive treatment of patients with immune mediated inflammatory diseases affected the response to mRNA vaccines. Here I highlight some of the results from these studies and lessons learned. For example, SARS-CoV-2 mRNA vaccines induced IL-4, an important T cell cytokine that promotes long lived B cell responses, whereas we did not detect IL-4 upon restimulation of T cells from COVID-19 convalescent patients. Additionally, SARS-CoV-2 specific T cells from COVID-19 convalescent patients produced TNF upon restimulation, whereas those from vaccinated patients did not. This suggests that the T cell response to SARS-COV-2 infection is more inflammatory and less helpful to B cells than the T cell response to SARS-COV-2 vaccination. The number of vaccine doses required to induce maximal T cell cytokines differed depending upon the cytokine measured. Thus, measuring several cytokines can be important in determining the effects of boosting. Although T cell responses to SARS-CoV-2 provide important information to guide vaccine development, these assays are difficult to standardize across labs and more work is required in this area.

Abstracts

POSTER PRESENTATIONS

SYNTHESIS OF SMALL MOLECULE ANTIVIRAL AGENTS VIA METALLOCARBENE-AZIDE CHEMISTRY AND DOMINO NUCLEOPHILIC TRAPPING

Shivesh Baburam, Andrew Daszczynski, and Frederick G. West

Respiratory Syncytial Virus (RSV) is a Group V, non-segmented negative-sense RNA virus, where the infected cells fuse together to form a syncytium (single cell with multi nuclei). RSV can lead to severe lower respiratory infections in children in North America and is one of the leading causes of death of infants worldwide. Recently, Health Canada has approved the first vaccine for RSV from GSK, and while this a monumental step in the progress of therapeutic agents to treat or prevent RSV, this vaccine is limited to individuals 60 and over. Prior to the release of this vaccine, the only approved therapeutic was the prophylactic drug palivizumab, a highly expensive monoclonal antibody limited to high-risk individuals. The scarcity of therapeutics to treat and/or prevent RSV created an opportunity for us to design small molecule antiviral agents that may inhibit the viral RNA dependent RNA polymerase complex (RdRp), as it provides an attractive target for inhibition of the viral replication cycle. Utilizing novel metallocarbene-azide coupling to generate reactive C-acylimines, we can access unique scaffolds with various nucleophilic trapping partners that resemble the naturally occurring antiviral isatisine A.

MHC-I-EXPRESSING GENETICALLY ENGINEERED VACCINIA VIRUS AS A PRECISION MEDICINE CANCER VACCINE

Shae Komant, Jun Li Wang, Cyril Alex, Ryan S Noyce, Nicole Favis, David H. Evans and Troy A. Baldwin

Oncolytic viral therapy uses viruses to selectively infect and kill cancer cells, while inducing an anti-tumor immune response. The ability of current oncolytics to stimulate anti-tumor responses varies, therefore novel means of inducing immune activity is necessary. Vaccinia virus (VACV) is a promising candidate due to its established safety profile and ability to produce potent immune responses. Here, we use modified VACV expressing MHC-I with a defined peptide and ß2-Microglobulin as a single chain trimer (SCT). Additionally, the virus encodes the T-cell co-stimulatory molecule CD80. To determine if VACV-SCT, expressing a peptide from chicken ovalbumin (OVA), can induce antigen specific responses we infected murine melanoma B16F10 cells with VACV variants and co-cultured the infected tumor cells with OVA specific T-cells. Three days post co-culture T-cells expressed activation markers and proliferated, indicating VACV expressed peptide-MHC-I stimulates T-cell responses. We next determined if intratumoral injection of EMT6 breast cancer cells can lead to tumor regression in vivo. For the EMT6 model a previously described tumor specific antigen, E22, was expressed by the SCT. Following virus treatment, E22 specific CD8+ T-cells infiltrated the tumor and were present in the spleen. Approximately 70% of mice treated with VACV-SCTE22 cleared the tumor. Further, all mice that cleared primary tumor also cleared secondary tumor upon challenge. These data highlight the potential to generate a VACV stimulated immune response by intratumoral delivery of a defined peptide-MHC-I complex. This ability to bolster a T-cell response is important for the advancement of immunotherapies and VACV as a vaccine platform.

Research funded by the Li Ka Shing Institute of Virology and The Johnston Family Melanoma Research Program funded by the Mary Johnston Chair in Melanoma Research through the Alberta Cancer Foundation.

INTERFERING WITH INFLUENZA'S CAP-SNATCHING VIRAL POLYMERASE

Justine Beghin, Kira Sviderskaia, Kuldeep Kaur, Matthias Götte, Lorne Tyrrell, and Vanessa Meier-Stephenson

Influenza viruses make up a leading cause of pandemics, with an estimate of >1 billion infections occurring annually. Influenza virus is a multi-segmented, negative-stranded RNA virus, with many diverse strains. Each of its segments requires the RNA-dependent RNA polymerase (IAVpol) to undergo viral replication and transcription though the process of cap-snatching, where the 5′ caps of host mRNAs are used for the viral RNAs. These processes are highly conserved making them good therapeutic targets. Aptamers are short oligonucleotides that fold into distinct shapes and can bind selectively to targets to interfere with their function. Given their size and stability, aptamers are more druggable than antibodies, making them a possible method for probing novel target sites on IAVpol. To design a small molecule therapeutic to target Influenza A Virus polymerase using an aptamer-based approach. The IAVpol (wild-type and a known mutant) complex will be produced using the Spodopterafrugiperda 9 (Sf9) insect cell line and purified using affinity column chromatography. Next, the complex will be subjected to systematic evolution of ligands by exponential enrichment (SELEX) experiments, to determine aptamer hits that can bind IAVpol. Functional assays for in vitro replication and transcription of the IAVpol will be optimized to test aptamer hits and determine how aptamer binding impacts the function of IAVpol. Aptamers impacting either function of the IAVpol will undergo structural analysis with cryoEM for better characterization of the binding site and modality.Investigating how aptamer binding may interfere with IAVpol functioning may lead to novel therapeutic strategies for targeting influenza viruses.

WASTEWATER SURVEILLANCE OF INFLUENZA A AND RSV AND ITS CLINICAL CORRELATIONS IN EDMONTON, ALBERTA

Sudha Bhavanam, Judy Qiu, Bonita Lee, Nathan Zelyas, Emily Buss and Xiao-Li Pang

Wastewater-based surveillance (WBS) has shown promise in predicting COVID-19 cases caused by SARS-CoV-2, but its potential for monitoring other endemic respiratory diseases like influenza A (IAV), influenza B (IBV), and respiratory syncytial virus (RSV) remains underexplored. Our study aimed to assess WBS's ability to monitor respiratory diseases in Edmonton, Alberta, by correlating IAV and RSV RNA presence in wastewater with clinical testing data. Wastewater samples were collected from Edmonton's two wastewater treatment plants, serving a population of around 1.3 million, from January 2022 to September 2023. Following Centricon ultracentrifugation and RNA extraction, RT-qPCR assays quantified IAV, IBV, and RSV in the samples. Viral concentrations of IAV and RSV were then correlated with clinical cases within the Edmonton Health Zone, matching patient residence postal codes to the wastewater treatment plants' Forward Sortation Area (FSA). Among the 509 collected wastewater samples, 17.5% were positive for IAV, 2.6% for IBV, and 43% for RSV. In the period from January 2022 to January 2023, used for comparing WWS and clinical results, 23.6% were IAV positive, 0.3% IBV positive, and 51.2% RSV positive. Notably, RSV RNA concentrations in wastewater exceeded those of IAV and IBV ($p<0.001$). A moderate positive correlation between clinical cases and wastewater samples was established for IAV and RSV (r2 of 0.61 and 0.65, respectively, $p<0.001$). These findings underscore WBS's effectiveness in monitoring IAV and RSV, contributing to the prediction of clinically confirmed diseases in Edmonton. WBS emerges as a valuable, objective, and inclusive tool for tracking endemic respiratory viruses.

INVESTIGATING POTENTIAL ORTHOPOXVIRUS DRUG TARGETS UTILIZING DE NOVO DNA SYNTHESIS

Nolan Bird, Stephen Lee, David Evans and Ryan Noyce

Orthopoxviruses are large DNA viruses that include important human pathogens, such as the causative agents of smallpox and mpox. Due to the significant homology between members of the family, the study of a prototypical Orthopoxvirus, vaccinia virus (VACV), allows us to learn more about the underlying mechanisms of these pathogens. One essential aspect of the Orthopoxvirus lifecycle that has yet to be fully elucidated is assembly and encapsidation. Through investigation of this vital process, potential drug targets may be revealed. It is known that two hairpin binding proteins I1 and I6 are required for proper assembly and encapsidation. However, the precise mechanisms are not fully understood. Initially, a 6xHis-tag was introduced to the C-terminus of I6 within the viral genome, which would allow for expression and purification from infected cells. However, the recombinant virus was attenuated with lower titers and a small plaque phenotype. The his-tag likely resulted in misfolding and premature degradation of I6, since purification of the protein was unsuccessful. The next step is to clone I6 into a baculovirus expression system, in order to purify and study the protein. BioXP® technology will be used to synthesize the I6 gene de novo, and clone it into a bacmid. Purification of I6 allows for quantitative biochemistry experiments that will shine light on the process of assembly and encapsidation. Additionally, the de novo DNA synthesis technology can be utilized for high-throughput screening of different mutations which would be useful in antiviral drug development.

MECHANISTIC INSIGHTS INTO SMALL RIBONUCLEIC ACID MEDIATED GENE SILENCING VIA PROQ/FINO DOMAIN PROTEINS

Mazzen Black and J. N. Mark Glover

Small non-coding RNAs (sRNAs) have been demonstrated to be vital for the regulation of gene expression in living organisms, but they are often not stable alone. Protein cofactors aid and mediate interactions between sRNAs and mRNA targets, working to protect sRNAs from degradation. This can be accomplished through destabilizing the internal structures within RNA that can prevent interactions and complementary binding. sRNAs can impact the regulation of gene expression via repression by interacting with the Shine-Dalgarno sequence, translational activation by binding further upstream in the gene, or mRNA stabilization. The FinO protein family is a major class of chaperones in gram-negative bacteria and are responsible for regulating critical functions such as antibiotic resistance, stress response, and virulence. RocC (Repressor of competence Chaperone) is one example targeting the sRNA RocR (repressor of competence RNA). Together, this complex represses environmental DNA uptake by targeting the ComEA mRNA. NMB1681, another protein in this family, is an ideal protein to study as it represents an almost 'pure' FinO domain with no N- or C-terminal modifications, and previous research has presented a solved crystal structure and evidence that it acts as a global RNA binder with specific target recognition important to the overall health of the cell. Additionally, NMB1681 seems to bind to its target in a different manner than RocC, and appreciating the differences could yield a mechanism of binding. Understanding the mechanism of action is important due to N. meningitidis being a leading cause of meningitis and sepsis in humans.

Abstracts

SARS-COV-2 MPRO PROTEASE VARIANTS OF CONCERN DISPLAY ALTERED VIRAL SUBSTRATE AND CELL HOST TARGET GALECTIN-8 PROCESSING BUT RETAIN SENSITIVITY TOWARD ANTIVIRALS

Sizhu Amelia Chen, Elena Arutyunova, Jimmy Lu, Muhammad Bashir Khan, Shima Shahbaz, Jegan Iyyathurai, Eman Moussa, Zoe Turner, Bing Bai, Tess Lamer, James A. Nieman, John C. Vederas, Olivier Julien, Shokrollah Elahi, Howard S. Young, M. Joanne Lemieux

The main protease of SARS-CoV-2 (Mpro) is the most promising drug target against coronaviruses due to its essential role in virus replication. With newly emerging variants there is a concern that mutations in Mpro may alter the structural and functional properties of protease and subsequently the potency of existing and potential antivirals. We explored the effect of 31 mutations belonging to 5 variants of concern (VOCs) on catalytic parameters and substrate specificity, which revealed changes in substrate binding and the rate of cleavage of a viral peptide. Crystal structures of 11 Mpro mutants provided structural insight into their altered functionality. Additionally, we show Mpro mutations influence proteolysis of an immunomodulatory host protein Galectin-8 (Gal-8) and a subsequent significant decrease in cytokine secretion, providing evidence for alterations in the escape of host-antiviral mechanisms. Accordingly, mutations associated with the Gamma VOC and highly virulent Delta VOC resulted in a significant increase in Gal-8 cleavage. Importantly, IC50s of nirmatrelvir (Pfizer) and our irreversible inhibitor AVI-8053 demonstrated no changes in potency for both drugs for all mutants, suggesting Mpro will remain a high-priority antiviral drug candidate as SARS-CoV-2 evolves.

CRISPR TECHNOLOGY INCORPORATING AMPLIFICATION STRATEGIES FOR MOLECULAR DETECTION

Wei Feng, Hanyong Peng, Yanming Liu, Jianyu Hu, Huyan Xiao, Jingyang Xu, Graham Tipples, Michael A. Joyce, D. Lorne Tyrrell, Shawn Babiuk, Hongquan Zhang and X. Chris Le

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) protein systems have transformed the field of genome editing. Progress in CRISPR–Cas technology has also advanced molecular detection. Our research aims at improving the specificity of genome editing (minimizing the off-target effect), understanding the kinetics of the CRISPR nuclease activities, and developing CRISPR techniques for analytical applications. Incorporating CRISPR–Cas systems with various nucleic acid amplification strategies enables the generation of amplified detection signals, enrichment of low-abundance molecular targets, improvements in analytical specificity and sensitivity, and development of point-of-care (POC) diagnostic techniques. These systems take advantage of various Cas proteins for their particular features, including RNA-guided endonuclease activity, sequence-specific recognition, and multiple turnover trans-cleavage activity of Cas12 and Cas13. We have successfully integrated reverse transcription (RT), recombinase polymerase amplification (RPA), and CRISPR-Cas12a nuclease reactions for sensitive fluorescence detection of SARS-CoV-2 RNA. We have also developed colorimetric assays by integrating CRISPR technology with loop-mediated isothermal amplification techniques. With these integrated strategies, nucleic acid amplification ensures the sensitivity of molecular assays, and the use of the CRISPR–Cas system after nucleic acid amplification improves detection specificity due to RNA-guided recognition of specific sequences of amplicons. To speed up the analysis, we have studied the kinetics of CRISPR-Cas activation and its nuclease activities. The insight gained from the fundamental understanding enabled us to design rapid CRISPR-based diagnostic assays that operate at the ambient temperature. These assays are applicable to the detection of diverse nucleic acid targets.

DEVELOPING MAMMALIAN ORTHOREOVIRUS AS A RECOMBINANT VACCINE VECTOR

Tim Footz, Heather Eaton, Shae Komant, Maia Finch, Troy Baldwin and Maya Shmulevitz

Research with recombinant viral vectors is an important facet of the development of vaccines, which are effective tools to address public health during epidemics and pandemics. Current viral vector candidates display positive performance with regards to low virulence, rapid re-engineering to combat emerging variants, thermostability, high immunogenicity and long-lasting protection. These benefits drive the need to evaluate more viral vectors to increase the options available for development of vaccines for outbreak preparedness. We propose that mammalian orthoreovirus, a ubiquitous enteric virus with low pathogenicity, will be a safe and effective viral vector. Using a prime-boost approach in murine models with the prototypic lab strain T3DPL, we compared different routes of virus administration: oral, intranasal and intraperitoneal. The methods of analysis utilized serum, intestinal lavage, fecal samples and primary splenocytes to characterize humoral antibody production and T-cell response. Our data demonstrate that inoculated mice generated serum and mucosal antibodies (IgG and IgA isotypes) against the sigma1 protein (encoded by the S1 gene), which is responsible for cell attachment and is a favourable target for expression of heterologous viral antigens. Our flow cytometry results also show that reovirus-specific T-cell activation was achieved. As a step towards evaluating the utility of reovirus as a recombinant vector, we used an efficient reverse genetics system to generate viruses expressing either fused or cleaved norovirus antigens at a truncated S1 locus. We anticipate that these constructs will be helpful in establishing the value and validity of using reovirus as an easily modified vector for vaccine development.

IDENTIFICATION OF HOST DEPENDENCY FACTORS IMPORTANT FOR THE REPLICATION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 VARIANTS OF CONCERN IN VITRO AND IN VIVO

Alberto Felix-Lopez, Mohamed Elaish, Joaquin Lopez-Orozco, Nawell Fayad, Zack Xu, Anil Kumar, and Tom C. Hobman

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is responsible for the current COVID-19 global pandemic. The common symptoms of COVID-19 are fever, dry cough, and tiredness. However, in severe cases, difficulty breathing, chest pain or pressure, loss of speech or movement can result in death. There are almost 700 million cases and more than 6 million deaths worldwide.Multiple VOCs (Variants of Concern) have spread throughout the pandemic faster than the previous dominant variant. The most prevalent variant is Omicron, which was preceded by the Delta and Alpha VOCs. Understanding how SARS-CoV-2 interacts with the host cell is essential for developing novel vaccines and therapeutics. We employed a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) – Knockout screen in Human embryo kidney cells stably expressing ACE2 (Angiotensin I Converting Enzyme 2). We identified several host dependency factors for multiple VOCs. Using multiple FDA approved chemical inhibitors, we validated the importance of several host dependency factors during the viral infection in HEK293T-ACE2 and in primary human cells. These host factors are highly involved in numerous cellular processes including cell proliferation, survival, differentiation, migration, hematopoiesis, cell homing and ubiquitination pathways. We are currently exploring the specific viral proteins being affected by the disruption of these host dependency factors, in which step of the infection they are involved in, their importance in the replication of other RNA viruses and validating their importance during the infection of mice.

OPTIMIZATION AND EFFICACY ANALYSIS OF STRUCTURE-BASED PRION VACCINES IN TRANSGENIC MOUSE MODELS

Caleb Duckering, Madeleine Flemming, Andrew Fang, and Holger Wille

Prion diseases are fatal neurodegenerative disorders caused by the misfolding of the cellular prion protein, PrPC, into an infectious conformer, PrPSc. The structure of PrPSc is still a subject of debate within the scientific community. There have been numerous cryo-EM structures published in recent years that give support for a parallel in-register intramolecular β-sheet (PIRIBS) conformation. However, there is also evidence indicating that the structure of PrPSc could adopt a four-rung β-solenoid fold. Vaccine candidates based on the PIRIBS structures and the β-solenoid model have been created using an innocuous fungal protein, HET-s. This protein readily forms amyloid fibrils and was selected as a scaffold to present a variety of antigen epitopes. Three PIRIBS-based and one β-solenoid model-based vaccine candidates have been created to target PrPSc. Additional vaccine candidates will be created using a soluble scaffold protein: pectate lyase-3 (PL3). To test the efficacy of our vaccine candidates two mouse lines have been selected, TgElkPrP and TgP101L. These transgenic mouse lines help test the vaccine's effectiveness in protecting against PrPSc. The trials using TgP101L mice injected with the β-solenoid-based antigen, 14R1, have resulted in increased longevity and delayed symptom onset compared to control groups. Current experiments with TgP101L mice show low efficacy for the PIRIBS-based vaccine candidates. Although the mice display a strong immune response, their health and survival status show minimal to no improvements compared to control groups. Trials using TgElkPrP mice to test the vaccine candidates against a cervid prion disease known as chronic wasting disease (CWD) are ongoing.

RATIONALLY DESIGNED, STRUCTURE-BASED VACCINE CANDIDATES TARGETING CHRONIC WASTING DISEASE

Andrew Fang, Xinli Tang, Brian Tancowny, Peach Van Wick, Samantha Allen, Nathaniel Denkers, Candace Mathiason, Holger Wille

Chronic wasting disease (CWD) is the most contagious prion disease, affecting free-ranging and captive cervids, including deer, elk, and moose. No cures or prophylactic vaccines exist for any prion disease including CWD, resulting in an invariably fatal outcome for an infected host. Based upon a four-rung β-solenoid model of PrPSc, our lab has developed a protocol using a modified fungal prion protein as an innocuous scaffold for the rational design of structure-based vaccine candidates targeting CWD. The alternating position of amino acid side chains within the β-strands (inwards versus outwards facing) was used to strategically place predicted surface residues of PrPSc onto the surface of the scaffold. The resulting vaccine was used to immunize mice that spontaneously developed a genetic prion disease and demonstrated efficacy in prolonging the lifespan. We then immunized both elk and deer in Wyoming and Colorado, respectively. The immunized elk developed PrPSc-specific immune responses in 75% of the animals, while non-immunized animals had no measurable titer. The immunized deer have thus far shown a measurably increased antibody titer compared to non-immunized animals, and the vaccine was well tolerated in both species. A monoclonal antibody derived from the spleens of immunized mice was shown to preferentially recognize human and animal prion disease strains over their non-infectious counterparts using competition ELISAs. Future directions include vaccine optimizations by using different scaffold proteins and to elicit a mucosal immunoglobulin A response for the vaccination of cervids against oral CWD exposure.

Abstracts

RECENT ADVANCES IN THE SYNTHESIS OF 2-(3-INDOLYL)INDOLIN-3-ONE ANTIVIRALS

Israel Najar Guerrero, David J. Marchant and Frederick G.West

RNA viruses have been the main cause of epidemics over the last twenty years, some examples are: SARS-Cov-1 in 2003, Influenza A subtype H1N1 in 2009, MERS-Cov in 2012, Ebola virus in 2014, Zika virus in 2015, and most recently, SARS-Cov-2 2019. All these viruses require the RNA - dependent RNA polymerase complex (RdRp) as a key enzyme during the RNA replication process. The active site of RdRp is highly conserved among RNA virus, leading to the identification of this protein as a promising target for broad spectrum antiviral drugs. Another advantage of targeting RdRp is the lack of host homolog protein, therefore; there is a reduced risk that proteins in human cells will be affected by RdRp inhibitors. 2-(3-Indolyl)indolin-3-one analogues of the natural product isatisine A ,developed by the West and Marchant groups, are RdRp inhibitors that have showed promising antiviral activity against Respiratory Syncytial Virus and Zika virus. In this work, we present the recent advances in the diversification of the small molecules library with the incorporation of different β-dicarbonyl moieties in order to evaluate different biological responses and the improvements in the synthesis process replacing the use of potentially dangerous diazo intermediates with the use of the more benign iodine (III) compounds allowing a safer scale up synthesis of the antiviral molecules.

OPTIMIZING CAS12A SPECIFICITY THROUGH INTUITIVELY DESIGNED HAIRPIN MODIFIED CRISPR-RNA

Camille Huang, Jianyu Hu, Wei Feng, Hongquan Zhang and X. Chris Le

Newly developed Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) detection techniques have been used for the detection of specific nucleic acid sequences. However, current CRISPR-based detection techniques are difficult to differentiate single-nucleotide mutations. To address this problem, researchers have attempted to modify the CRISPR-associated (Cas) protein or manipulate target complementary CRISPR (cr)-RNA. Our research focuses on designing and modifying crRNA, for example, by extending crRNA at the 3′ end to form a hairpin secondary structure along the spacer region. We have designed hairpin (Hp)-crRNAs containing 8, 10, 12, 13, and 14 nucleotide stem extensions. The introduction of hairpin structure in the crRNA enables control and modulation of the binding and Cas activation free energy. We harness the energy difference between the wild-type and single-nucleotide mutation to achieve differentiation of them by the CRISPR system. As an example, we are developing a CRISPR technique, using LbCas12a, for the detection and differentiation of a vancomycin-resistance single-nucleotide mutation. The technique has potential to be used for the detection of drug-resistant genes, new variants of infectious agents, and single nucleotide polymorphisms (SNP). Manipulation of the crRNA to improve specificity should also contribute to minimizing the off-target effect in CRISPR technology.

MONITORING OF SARS-COV-2 VARIANTS IN WASTEWATER USING WHOLE GENOME SEQUENCING

Linnet Immaraj, Melissa Wilson, Qiang Jiang, Yuanyuan Qiu, Bonita Lee, Xiaoli Pang

Monitoring SARS-CoV-2 in municipal wastewater (WW) has been proven to provide valuable information on the prevalence of COVID-19 in a community and guide public health decision-making. However, this approach lacks tracking the spread and turnover of various emerging SARS-CoV-2 variants of concern (VOC). Our study aims to estimate the co-circulation of the VOC and its lineages in WW across Alberta using whole-genome sequencing (WGS) and computational techniques. WW samples were collected thrice weekly from eleven wastewater treatment plants, serving a population of approximately 3 million from July 2022 to June 2023. After centricon ultrafiltration and RNA extraction, the N1 and N2 genes were quantified using well-established RT-qPCR assays. We selected samples with the highest viral load per site weekly and prepared the WGS library using the ARTIC amplicon panel with the Illumina® DNA prep workflow. Subsequently, the pooled and cleaned library was sequenced on an Illumina® MiniSeq, and the Freyja tool was employed to estimate variant lineage abundances. Sequence datasets with SARS-CoV-2 genome coverage greater than 95%, and ~2 million paired-end reads from 518 samples were used for data analysis. Our data shows that the average percentage abundance in Omicron variant lineages for all sites was observed to be BA.5 (91%), BQ.1 (84%), and XBB.1.5 (79%), and peaked during July 2022, Dec 2022, and Mar 2023, respectively, broadly reflecting the overall SARS-CoV-2 clinical trends in the community over this period. Thus, WW sequencing can be a powerful and effective tool to monitor trends in variant prevalence and viral genetic drift.

INHIBITION OF SARS-COV-2 BY PEPTIDE-BASED INHIBITORS

Michael A. Joyce, Holly A. Saffran, Justin A. Shields, Ulrike Strunk, Bing Bai, Appan Srinivas Kandadai, Elena Arutyunova, Matthew Croxen, Kanti Pabbaraju, Graham Tipples, M. Joanne Lemieux, James A. Nieman, and D. Lorne Tyrrell

The COVID-19 pandemic from Jan 2020 to May 2023 resulted in approximately 700 million SARS-CoV-2 infections and 7 million deaths. Despite the effectiveness of the initial vaccines, SARS-CoV-2 continues to evolve new variants that are poorly neutralized, resulting in new infections and outbreaks. SARS-CoV-2 is a positive strand RNA virus that codes for 2 polyproteins that are essential for viral replication as well as proteins produced from subgenomic RNAs. Cleavage of the polyproteins is carried out primarily by the main protease (Mpro). In collaboration, we have carried out antiviral testing of over 150 novel compounds, investigating the metabolic, enzymatic, and virologic effects of changes in the warhead, P2 and P3 positions of peptide based inhibitors of SARS-CoV-2 Mpro. Here, we will show inhibition of wild type and variant SARS-CoV-2 infection by inhibitors in cell lines and mice, as well as development of resistance to selected inhibitors by SARS-CoV-2, and the time of addition studies confirming that inhibitors act when expected during viral replication. Taken together these results show the development of effective inhibitors of SARS-CoV-2.

PROTEASE-ACTIVATED RECEPTOR 2 AGONIST AS MUCOSAL (LUNG) ADJUVANT

Gang Zhou, Harissios Vliagoftis and Kevin Kane

Current vaccinations against respiratory viruses such as influenza A (IVA) are almost exclusively delivered intramuscularly. Although this results in circulating antigen (Ag) specific antibodies and Ag specific T cells, the T cells in particular, do not enter lung tissue and set up residence as T effector memory cells (Tem) to combat exposure to the virus at the site of entry. We find intranasal delivery of IVA virosomes as a source of Ag, but lacking IVA nucleic acid, combined with a short peptide that activates the Protease-Activated Receptor (PAR-2) expressed by alveolar epithelial cells and monocyte derived cells in lung, is a very effective vaccination strategy, which results in substantially improved survival/protection of mice from intranasal infection by IVA. The inclusion of PAR-2AP (PAR-2AP-activating peptide) acts as a mucosal adjuvant increasing Ag specific Ab responses, and Ag-specific Tem in lung that are functionally competent in response to virus. Protection from IVA infection is transferred to naïve mice by lung Tem, originally induced following intranasal vaccination with PAR-2AP and virosomes in previous naïve mice. Intranasal delivery of virus Ags in combination with PAR-2AP may be a universally effective vaccination approach to establish effective memory B and T cells, especially in the lung where infection starts. There may be application of PAR-2AP as an adjuvant in mucosal (lung) vaccines against other respiratory viruses such as Respiratory Syncytial virus (RSV) and SARS-CoV2.

FUNCTIONAL ROLE OF DUCK RIPLET IN MODULATING ANTIVIRAL IMMUNE RESPONSES

Mirzabek Kazbekov, Katharine Magor

This study elucidates the role of duck RIPLET (Really Interesting New Gene [RING] Finger Protein 135) in avian antiviral immune responses, specifically its interaction with the Retinoic Acid-inducible Gene I (RIG-I). The resistance of ducks to Influenza A virus (IAV) is associated with the RIG-I receptor, while chickens, lacking RIG-I, are more vulnerable to IAV infections. The controversy in RIG-I activation centers on two ligases: RIPLET and Tripartite motif containing protein 25 (TRIM25), their specific ubiquitination targets, and the conflicting findings in various species, leading to an unresolved understanding of RIG-I activation mechanisms. Research has predominantly concentrated on RIG-I activation by primarily examining the isolated N-terminal domain of RIG-I and stimulating it with TRIM25. Furthermore, the functionality of RIPLET in ducks was questioned because of the initial absence of the catalytic RING domain based on previously available genome. Our research not only confirms the presence of the RING domain in duck RIPLET but also explores its pivotal role in avian antiviral immunity. Importantly, we leverage our unique advantage of using chicken DF-1 cells, devoid of endogenous RIG-I and RIPLET, allowing precise control over their levels. Employing transfection techniques, dual luciferase assays, quantitative PCR, and co-immunoprecipitation, we investigate how duck RIPLET influences IFN-β promoter activity, enhances antiviral immunity, and interacts with RIG-I in these cells. Studying this in a comparative system can help us understand the functions of these E3-ubiquitin ligases in RIG-I activation across various species, thereby enhancing our understanding of universal mechanisms in antiviral immunity.

Abstracts

IDENTIFYING DOMAINS IN REOVIRUS STRUCTURAL PROTEINS Λ2 AND Σ1 AMENABLE TO GENETIC MANIPULATION

Justine Kniert, Heather E. Eaton, and Maya Shmulevitz

Mammalian orthoreovirus (reovirus) is minimally pathogenic in nature, thus lending itself as a promising vaccine vector candidate. However, incorporating exogenous genes into the small dsRNA genome of reovirus without compromising on the virus' ability to infect and express the gene of interest remains challenging. A deeper understanding of the reovirus protein structure-function relationship is required to use the virus as a molecular delivery tool. Using a structural analysis approach with published Protein Data Bank files, we identified domains in the λ2 core protein that could be amenable to manipulation. Of domains identified, only one was capable of supporting tag (FLAG, HA, or V5) incorporation using conventional molecular cloning techniques. A similar approach was taken with the outercapsid σ1 protein, resulting in the successful generation of a recombinant reovirus containing a FLAG-tagged λ2 and a Myc-tagged σ1. Our ongoing investigation aims to determine which σ1 domains are required for proper protein localization and virion assembly within infected cells. A series of σ1 truncation mutants are being generated in fluorescently-tagged plasmids for transfection based experiments assessable by immunofluorescence confocal microscopy in the context of viral infection. Through our work, we aim to gain a deeper understanding of reovirus protein structure-function that will allow us to better manipulate reovirus as a vaccine vector.

MYELOID SUBSETS MODULATION FOLLOWING PAR-2 ACTIVATING PEPTIDES PRIMING IN AN INFLUENZA A VIRUS VACCINE AT AN EARLY STAGE IN MICE

Claudia Kornuta, Gang Zhou, Kevin Kane and Harissios Vliagoftis

Protease-Activated Receptor 2 (PAR-2), a G protein-coupled receptor, is activated by serine proteases involved in various cellular processes. PAR-2 can be activated by synthetic peptides known as PAR-2 activating peptides. We have developed a new strategy for mucosal vaccination against the Influenza A Virus in mice using this novel activating peptide (AP) as adjuvant to enhance cell-mediated immunity. Previously, we observed that AP improved protection against subsequent lethal IAV infection (PR8) when co-delivered intranasally with virosomes in a mouse prime-boost vaccination model. However, the mechanism of action of AP adjuvant function is unknown. We hypothesize that myeloid cells recruitment to the lung and lymph nodes after vaccination is mediated by the adjuvant effect. In a study involving WT C57BL/6 mice, we assessed recruitment of lung myeloid cell subtypes at 3 and 14 days post-intranasal vaccination (dpv) with AP (25µL, 100µM) or virosome alone (600 Hemagglutinin unit), AP+Virosome, PBS or inert peptide (CP) as controls. At 3dpv, we detected differences in pro-inflammatory subset populations in mice treated with AP alone, including eosinophils and NK cells ($p<0.05$), and differences in the monocyte population in AP and Virosome+AP groups ($p<0.05$), specifically regarding inflammatory monocytes in the latter group ($p<0.01$) versus controls. However, no differences were found in different subset populations at 14dpv. These preliminary results suggest that AP induces an early pro-inflammatory environment, potentially leading to improved antigen presentation of Influenza virosomes to antigen-presenting cells. Further analysis including the inflammatory environment in draining LN is needed to determine which transcription factors are involved.

DEVELOPMENT OF VACCINE CANDIDATES AGAINST PLACENTAL MALARIA USING PEPTIDE-DECORATED ANTIGENIC LIPOSOMES

Payton LeBlanc, Dr. Daniel Ferrer Vinals, Edward Schmidt, Dr. Maju Joe, Dr. Matthew Macauley, Dr. Stephanie Yanow

The WHO reported over 13 million pregnancies exposed to malaria infection in 2021, yet there is currently no vaccine available against placental malaria, placing both the mother and fetus in danger. Plasmodium falciparum causes placental malaria by infecting red blood cells that accumulate in the intervillous space of the placenta. Infected cells display the parasite virulence factor VAR2CSA that binds to chondroitin sulfate A (CSA) chains present on the placenta. The sequestration of infected red blood cells (iRBC) triggers inflammatory responses with deleterious effects on the mother and the fetus. We identified two VAR2CSA-derived peptides, P6 and P10, that induced functional antibodies that prevented binding of iRBCs to CSA in vitro. This presents an opportunity to develop a novel peptide-based vaccine against placental malaria. In this project, we developed multivalent peptide-decorated liposomes to optimize the immunogenicity of P6 and P10. Initially, we employed copper-free azide-alkyne click chemistry to prepare the conjugated lipopeptides. Using thin film hydration we prepared peptide-decorated liposomes with an average size of 250 nm and with controlled polydispersity. Rat antisera against P6 and P10 strongly recognized both peptides on the liposomes. In next steps, we will immunize mice with the peptide-decorated liposomes and evaluate their immunogenicity and the functional activity of antibodies against placental iRBCs. Our ultimate goal is to develop multi-epitope vaccines by incorporating P6 and P10 onto the same lipid nanoparticle, which could lead to a more potent vaccine candidate against placental malaria.

MECHANISMS OF RESISTANCE TO REMDESIVIR ASSOCIATED WITH SARS-COV-2 CLINICAL ISOLATES

Hery W. Lee and Matthias Götte

Remdesivir (RDV) is a small-molecule antiviral drug approved by the US Food and Drug Administration for treatment of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Specifically, RDV-triphosphate (-TP) is a nucleotide analogue mimicking the natural ATP. Its most notable modification is a cyano-moiety at the 1' position of the ribose ring responsible for the drug's primary modes of action. We have previously determined that SARS-CoV-2 selectively incorporates RDV-TP over ATP during RNA synthesis. Here, the viral polymerase incorporates RDV, which in turn causes a steric clash that inhibits elongation in a process termed "delayed chain termination." Eventually, this results in RNA strands that have RDV in their sequence, later able to be used as a template by the viral polymerase. Templated RDV then acts through a second mode of action by stalling synthesis through steric hindrance. Due to its efficacy and to limited treatment options available, it is critical to understand how viral resistance to RDV is promoted and prevented. Studies in vitro and in the clinic have already identified an important mutation in the SARS-CoV-2 polymerase that confers such resistance: a Val-to-Ile substitution in residue 792 of the polymerase's non-structural protein 12. Here, we biochemically characterize the mechanisms of resistance conferred by this substitution by examining enzyme kinetics in the SARS-CoV-2 polymerase and the homologous murine hepatitis virus polymerase. Our findings demonstrate that the mutation promotes ATP incorporation and, more importantly, grants the ability to overcome inhibition by templated RDV in both models.

DUCK LONG NONCODING RNAS AS POTENTIAL RIG-I INTERACTORS IN INFLUENZA A VIRUS INFECTIONS

Renald James Legaspi and Katharine Magor

Long noncoding RNAs (lncRNAs) are RNA molecules over 200 nucleotides long which have garnered attention for their essential cellular functions despite lacking protein-coding capabilities. LncRNAs play critical roles in immune responses, operating through diverse mechanisms as signals, decoys, guides, or scaffolds. LncRNAs have emerged as significant regulators of influenza A virus (IAV) infection, influencing viral replication, pathogenicity, and immune response. Ducks are the natural host and reservoir of IAV. Unlike chickens, ducks possess an RNA-sensing receptor, RIG-I, which plays a pivotal role in detecting "non-self" RNAs, activating antiviral proteins, and interferon-stimulated genes (ISGs). In mammalian systems, lncRNAs modulate the RIG-I signaling pathway during viral infections. Although the roles of lncRNAs in IAV have been demonstrated in mammals, their impact in ducks remains poorly understood. Here I screen duck lncRNAs that are potential ISGs and interactors of duck RIG-I, modulating antiviral innate immune responses in highly pathogenic IAV infections (HPAI). I analyzed gene expression in HPAI-infected duck samples and used machine learning to identify four lncRNAs with potential to bind to RIG-I. I assessed candidate lncRNAs interaction with activated RIG-I on IFN-β promoter activity in chicken cells, and identified lncRNAs enhancing the promoter activity. To verify the physical association between duck RIG-I and these enhancing lncRNAs in vitro, I am performing RNA-Immunoprecipitation. This project will shed light on the potential regulatory role of lncRNAs in the immune response to IAV infection in ducks and contribute to our understanding of the host-virus interactions.

CHARACTERIZING LAB-ADAPTED AND NATURALLY CIRCULATING REOVIRUS VARIANTS FOR TRAITS BENEFICIAL TOWARDS ORAL VACCINE DEVELOPMENT

Qi Feng Lin, Casey Wong, Heather E. Eaton, Xiaoli Pang, Maya Shmulevitz

Reovirus infects the gut of all mammals without causing disease, yet induces a potent immune response, making it a promising oral vaccine candidate. However, reovirus studies are primarily based on laboratory strains adapted to non-enteric cell culture models. Studying naturally circulating reoviruses could reveal characteristics promoting enteric infection absent from lab-adapted strains; to investigate this possibility, four genetically distinct "wild" reovirus isolates were obtained from the Edmonton sewage system. In the L929 mouse fibroblast cell model commonly used in reovirus research, wild reoviruses produced smaller plaques than prototypic viruses, suggesting lower viral fitness. Comparison of different viral replication steps revealed that wild viruses have delayed outercapsid uncoating and de novo protein synthesis despite similar cell attachment. Pre-processing the outercapsid of wild viruses with intestinal lavage increased their infectivity more than lab-adapted strains, indicating that wild reoviruses benefit more from intestinal factors for infection. To further investigate the delayed uncoating, wild and lab reoviruses were digested in vitro using endosomal and intestinal proteases. Most digestive enzymes processed lab-adapted and wild viruses with similar kinetics, with a few exceptions among wild viruses pointing towards potential host tropism preferences. However, all wild reoviruses were more resistant to proteolysis by cathepsins than lab-adapted strains. Overall, my project highlights the genetic diversity of reovirus and identifies phenotypic differences between lab-adapted and wild reoviruses in cell culture. These results will allow us to test which molecular characteristics of lab-adapted and wild reoviruses are optimal for enteric infection and serve as oral vaccine.

Abstracts

IINVESTIGATING BALOXAVIR ACID AS A SCAFFOLD FOR THE DEVELOPMENT OF NOVEL CAP-SNATCHING ENDONUCLEASE INHIBITORS

Arlo Loutan, Egor Tchesnokov, and Matthias Götte

Segmented negative sense RNA (snsRNA) viruses rely on the conserved process of cap-snatching to initiate gene transcription. In cap-snatching, host 5' methyl-7-guanosine capped mRNA or pre-mRNA are cleaved, generating a 3' hydroxyl group that acts as a primer for viral transcription. The endonuclease domain of the RNA-dependent RNA polymerase (RdRp) is responsible for mRNA cleavage. The RdRp represents an optimal antiviral target due to its conservation across families and essential role in the viral replication cycle. The endonuclease of influenza virus RdRps is inhibited by baloxavir acid (BXA). Using its metal-chelating domain, BXA binds to the divalent metal cations in the endonuclease active site. The specificity domain of BXA further stabilizes binding through interactions with hydrophobic amino acids. This scaffold provides a rationale for exploring compounds possessing comparable functional domains, which are hypothesized to inhibit snsRNA endonucleases through a similar mechanism. Recombinant full-length RdRps of influenza A and B viruses, severe fever with thrombocytopenia syndrome virus, Hantaan orthohantavirus, and Sin Nombre orthohantavirus are expressed using the baculovirus expression vector system and purified. Potential inhibitors are screened using biochemical gel-based assays followed by characterization of inhibitory concentration and mechanism of action. As expected, BXA inhibits influenza B RdRp more efficiently than other snsRNA viral RdRps. Of the novel compounds, several compounds demonstrate inhibition at concentrations over 100 micromolar. These compounds will be further explored to elucidate structure-activity relationships and improve potency. Ultimately, a thorough understanding of the structure-activity relationship will support the development of novel antiviral therapeutics.

INTEGRATION OF PROTEOMICS IN VIROLOGY: BUILDING VIRUS-HOST PROTEIN INTERACTION NETWORKS

Shu Luo, Joaquin Lopez Orozco, Eman Moussa, Alberto Felix Lopez, Erik Gomez Cardona, Nawell Fayad, Anil Kumar, Tom C. Hobman, Olivier Julien

With the devastating global impact of the COVID-19 pandemic, health research in virology is proven to be vital to combat, prevent and to better prepare us against future viral outbreaks. To unravel the intricate molecular mechanisms of viral infections, we aim to study the virus-host protein-protein interactions using specialized proteomics techniques. In particular, we have characterized the proteolysis targets of SARS-CoV-2 proteases, and began to investigate the virus-host protein-protein interaction networks of emerging mosquito-borne alphaviruses. SARS-CoV-2 is the causative agent of the recent COVID-19 pandemic. It encodes two proteases, Mpro and PLpro, that are essential for viral replication. As viral proteases are popular targets for antiviral drug development, the identification of their host targets further reveals their functional role in disrupting cellular processes. We characterized over 200 potential host substrates of SARS-CoV-2 Mpro and PLpro in vitro using mass spectrometry (1). Modulating proteolysis of these substrates will improve our understanding of SARS-CoV-2 pathobiology and COVID-19. Another global threat comes from mosquito-transmitted viruses, infecting up to 700 million people and leading to one million deaths worldwide every year. The scarcity of effective vaccines and treatment puts us at risk of facing future viral outbreaks. Therefore, we expand our studies to define the interactomes of all viral proteins in two emerging mosquito-borne viruses, Chikungunya and Mayaro viruses (2). Currently, we are performing co-immunoprecipitation on infected cells, creating maps of host-virus protein interactions during the course of infection. Targeting key host factors could become new therapeutic avenues to treat mosquito-borne viral infections.

SCREENING FOR BROAD SPECTRUM INHIBITION OF HUMAN CORONAVIRUSES

Cassidy Maplethorpe, Pu Chen, Elana Arutyunova, Tess Lamer, Bing Bai, John C. Vederas, James A. Nieman, M Joanne Lemieux

In light of the current pandemic, with the evolving SARS-CoV-2 variants and the incessantly circulating common human coronaviruses, the need for drugs effective against a range of coronaviruses is increasingly urgent. The coronavirus main protease, Mpro, is an attractive drug target due to its central role in viral replication and high conservation across coronavirus. The urgency of the COVID-19 pandemic has led to significant research efforts aimed at identifying and developing Mpro inhibitors, one of which includes FDA-approved Paxlovid™ that contains the Mpro inhibitor Nirmatrelvir. We have previously generated several potent Mpro inhibitors, and ongoing research continues to explore new compounds. The current work aims to evaluate the efficacy of SARS-CoV-2 Mpro inhibitors with a range of human coronaviruses, including common cold CoVs, and identify inhibitors that are broadly effective. We have cloned, expressed, and purified the Mpros of six human coronaviruses, SARS-CoV-2, HCoV-OC43, HCoV-229E, HCoV-NL63, HCoV-HKU1 and MERS-CoV, and determined the enzymatic activities for each. We have several inhibitor scaffolds known to inhibit SARS-CoV-2 Mpro, including Nirmetralvir, and we have assessed their potency with Mpro's of HCoV-OC43, HCoV-229E, and HCoV-NL63. These Mpros will be developed into a high throughput screen for use with new inhibitor derivatives. The best performing inhibitors will then be crystalized in complex with the Mpro of each human coronavirus. Given the ongoing global concern regarding coronaviruses and the potential for future outbreaks, a high-throughput multiplex assay screen for coronavirus Mpro inhibitors could be an impactful endeavor.

MECHANISMS OF RESISTANCE TO REMDESIVIR ASSOCIATED WITH SARS-COV-2 CLINICAL ISOLATES

Hery W. Lee and Matthias Götte

Remdesivir (RDV) is a small-molecule antiviral drug approved by the US Food and Drug Administration for treatment of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Specifically, RDV-triphosphate (-TP) is a nucleotide analogue mimicking the natural ATP. Its most notable modification is a cyano-moiety at the 1' position of the ribose ring responsible for the drug's primary modes of action. We have previously determined that SARS-CoV-2 selectively incorporates RDV-TP over ATP during RNA synthesis. Here, the viral polymerase incorporates RDV, which in turn causes a steric clash that inhibits elongation in a process termed "delayed chain termination." Eventually, this results in RNA strands that have RDV in their sequence, later able to be used as a template by the viral polymerase. Templated RDV then acts through a second mode of action by stalling synthesis through steric hindrance. Due to its efficacy and to limited treatment options available, it is critical to understand how viral resistance to RDV is promoted and prevented. Studies in vitro and in the clinic have already identified an important mutation in the SARS-CoV-2 polymerase that confers such resistance: a Val-to-Ile substitution in residue 792 of the polymerase's non-structural protein 12. Here, we biochemically characterize the mechanisms of resistance conferred by this substitution by examining enzyme kinetics in the SARS-CoV-2 polymerase and the homologous murine hepatitis virus polymerase. Our findings demonstrate that the mutation promotes ATP incorporation and, more importantly, grants the ability to overcome inhibition by templated RDV in both models.

DE NOVO DESIGN AND DISCOVERY OF ALLOSTERIC VIRAL POLYMERASE INHIBITORS

Ahmed K. Oraby, Leanne Bilawchuk, Egor P. Tchesnokov, Matthias Götte, Frederick G. West and David J. Marchant

Respiratory viruses are the most common cause of disease in humans causing high rates of death worldwide. Among these, Respiratory Syncytial Virus (RSV) is a major cause of severe lower respiratory infections for which effective treatment options remain limited. Moreover, the emergence of SARS-CoV-2 has created an even more urgent need for the development of new antiviral drugs. Herein, we employed a computational structure-based design strategy aimed at identifying potential targets for a new class of allosteric inhibitors. Our investigation led to the discovery of a previously undisclosed allosteric binding site within the RSV polymerase, the large (L) protein. The location of the site was supported by in vitro resistance assays, resistant virus sequencing, and structure activity relationship development of new generations of compounds. Moreover, A combination of virtual screening and molecular dynamics simulations enabled the design of a library compounds with enhanced potency. By understanding the binding pocket shape and binding requirements, we developed the next generation of inhibitors using a rational drug design approach. These findings hold promise for the development of versatile antiviral compounds with strong chemical and biological properties, potentially offering a solution for treating and containing RSV, SARS-CoV-2, and other respiratory infections.

SARS-COV-2 ACCESSORY PROTEIN ORF7B: A REGULATOR OF INTRACELLULAR CALCIUM TRANSPORT

Joseph Primeau, Christiane Bilodeau, Malana Loxam, and Howard Young

SARS-COV-2, the virus that causes COVID-19, remains a global priority. Though the primary route of infection and tissue damage occurs in pulmonary tissue, a significant patient population is subject to cardiovascular (CV) injury during disease progression. Amongst hospitalized patients, 20-30% exhibit some form of CV damage, where the severity of injury usually correlates with the severity of illness and can significantly increase morbidity and mortality. Infection likely occurs through endogenously expressed ACE2 receptors in cardiomyocytes, the primary target for SARS-COV-2 infection. Open reading frame 7b (Orf7b) is a 43-residue accessory protein in the SARS-CoV-2 genome. Orf7b's structure remains unknown; however, it likely folds similarly to the endogenous cardiovascular regulatory protein of the SarcoEndoplasmic Reticulum Calcium ATPase (SERCA): phospholamban (PLN). Previous efforts have identified that Orf7b is localized to the SR/ER. Orf7b was identified as a potential SERCA regulator because of its sequence similarity to the canonical SERCA regulator PLN. To investigate how Orf7b interacts with and modulates SERCA's calcium transport properties, recombinant Orf7b was expressed, purified, and coreconstituted with SERCA into proteoliposomes, serving as an experimental platform to assay ATPase activity and calcium transport. The results of these experiments suggest that Orf7b decreases SERCA's apparent calcium affinity. The results of this study indicate that an Orf7b – SERCA interaction could be a mechanism for cardiac injury in patients afflicted with COVID-19. Further research investigating targeted mutagenesis and binding models for Orf7b could lead to alternative therapeutic strategies for patients and drug development.

Abstracts

IDEUTERATION FOR METABOLIC STABILIZATION OF SARS-COV-2 INHIBITORS

Tayla Van Oers, Alexia Piercey, Alexandr Belovodskiy, Béla Reiz, Bethan Donnelly, Wayne Vuong, M. Joanne Lemieux, James Nieman, Karine Auclair, and John Vederas

Nirmatrelvir and GC373 are inhibitors of the SARS-CoV-2 main protease (Mpro) and prevent viral replication. Nirmatrelvir, the viral inhibitor in Pfizer's therapeutic, Paxlovid™, has been shown to be metabolized quickly by cytochrome P450 3A4 (CYP3A4). Therefore, ritonavir is also administered to act as a CYP3A4 inhibitor. However, this causes severe drug-drug interactions, as ritonavir blocks the metabolism of not just nirmatrelvir, but approximately 50% of all clinical therapeutics. To avoid this significant problem, a single-agent therapeutic is required, one that is at least as effective as nirmatrelvir, but more metabolically stable. The primary location of oxidation of nirmatrelvir by CYP3A4 is at the nitrogen adjacent methylene of the lactam ring. Our approach to preventing oxidation of this position was to install deuterium atoms in place of the hydrogen atoms. Carbon-deuterium bonds are stronger than carbon-hydrogen bonds and should slow the rate of oxidation at these positions. Key positions were deuterated in nirmatrelvir and GC373 and each analogue subjected to the CYP3A4 enzyme to determine oxidation levels by LC-MS. Deuterated nirmatrelvir was found to have oxidation reduced by about 40% compared to the non-deuterated compound. Deuterated GC373 appeared to have increased oxidation compared to the original non-deuterated GC373, and this surprising result is possibly due to a switch in the site of oxidation to the aldehyde warhead. The oxidation of the aldehyde warhead was detected and then confirmed by using LC-MS/MS analysis methods.

DISTINCT INHIBITION MECHANISMS OF 1'- AND 4'-CYANO MODIFIED ADENOSINE ANALOGUES AGAINST FLAVIVIRUS RNA POLYMERASES

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Flaviviruses are arthropod-borne RNA viruses that are recognized as important human pathogens. Dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), and Zika virus (ZIKV) provide prominent examples of viruses causing severe illness in humans. Currently, no approved antiviral treatments are available. Remdesivir (RDV) is a 1'-cyano modified C-adenosine monophosphate prodrug targeting the RNA-dependent RNA polymerase (RdRp) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was the first antiviral drug to receive approval from the US Food and Drug Administration (FDA) to treat COVID-19. As demonstrated in cell culture, RDV displays broad-spectrum antiviral activity, including DENV, JEV, WNV, YFV, and ZIKV. However, the mechanism by which RDV inhibits viral replication of these viruses remains unknown. ATP* is a newly discovered 4'-cyano modified C-adenosine analog that is likewise associated with broad- spectrum antiviral activity and an unknown mechanism of action. Here, RDV-TP is biochemically characterized in parallel with ATP* against the flavivirus RdRp. ATP* is incorporated more efficiently than RDV-TP. RDV-TP displays no inhibitory effect upon incorporation in the primer-strand, whereas ATP* incorporation results in immediate chain termination. Investigation of RNA synthesis opposite an embedded RDV revealed a template-dependent mechanism of inhibition that has not been previously described against flavivirus RdRp. These results exemplify the importance of efficient incorporation while illustrating the complementary mechanisms of a 1'- or 4'- modification on RNA synthesis inhibition when embedded in the template or during primer-strand synthesis, respectively.

TARGETING INSR SIGNALING TO MODULATE B CELL ACTIVATION AND FUNCTION TO TREAT OBESITY-RELATED COMORBIDITIES

Mengyi Zhu, Paulo José Basso, Sue Tsai, and Xavier Clemente-Casares

The low-grade chronic inflammation is a hallmark of obesity. This impaired inflammatory response predisposes to insulin resistance (IR) and is a gateway for multimorbidity and health complications. Current research has suggested that obesity is accompanied by impaired adaptive immunity elicited by vaccination and infection. It promotes us to investigate the mechanism underlying obesity-induced immune impairment. B cells contribute significantly to anti-viral immunity. Our previous data showed that B cells express insulin receptor (InsR), and sorted B cells from diet-induced obese mice exhibit impaired InsR signaling compared to those from lean mice. Thus, we aim to evaluate the impact of obesity and IR on B cells. We hypothesized that changes in B cell specific InsR signaling impair B cells function and can be normalized by metabolic modulation. Preliminary work found that InsR deficiency does not affect the relative proportions of bone marrow B cell precursors. Next, the immunization of B cell specific InsR knockout (InsRΔB) mice with ovalbumin (OVA)/CFA was used to define the effect of InsR deficiency on B cell activation and function, and we observed an impaired germinal center response and altered antibody class switching and production. Furthermore, we developed B cell-specific InsR deficiency mouse model in the context of influenza infection, and observed that InsRΔB mice exhibit increased vulnerability to H1N1 influenza infection compared to control mice. Our findings thus provide evidence of a regulatory role of InsR in B cell responses, highlighting its potential as target for future therapeutic interventions in obesity and other associated diseases.

TARGETING KEY REGULATORS IN BACTERIAL PATHOGENS: A PATHWAY TO PRECISE ANTIMICROBIALS

Julian Ortiz, Sarah Mansour, and Wael Elhenawy

There is an urgent need to develop new antimicrobials to combat multi-drug resistant bacteria. In addition to infections secondary to COVID-19, Alberta has experienced frequent bacterial outbreaks driven by Enterobacterales such as Shigella and Escherichia coli. Not only our current arsenal of antibiotics is facing rising levels of drug resistance among bacteria, but also the lack of selectivity is usually detrimental to the host microbiome. It is established that the microbiome is cardinal to human health, which includes promoting key immune functions. Therefore, improving the selectivity of novel antimicrobials is a top priority for the drug development pipelines. One promising strategy is to develop drugs that can disarm pathogens from their fitness advantage in vivo. Several studies showed that this can be achieved by targeting key regulators in these pathogens. We confirmed these findings by tracking the evolution of pathogenic E. coli in vivo, identifying a regulatory node of secondary metabolism that is critical for fitness. Informed by these findings, we developed a drug screening pipeline that can identify inhibitors of a key regulator in Enterobacterales. Importantly, our approach merged computational mining and empirical drug screening to expedite discovery and minimize the ensuing costs. This approach identified several compounds that can inhibit bacterial growth under infection-relevant conditions. Additionally, these compounds displayed activity against several pathogens under infection-relevant conditions. Future work will include the biochemical characterization of the identified drugs. Moreover, we will use our established animal infection models for the preclinical testing of the identified antimicrobials. This study provides a comprehensive framework for novel drug discovery.

INSIGHT INTO NUCLEOSOME ARCHITECTURE IN ABSENCE AND PRESENCE OF BRCTS

Rashmi Panigrah, Ross Edwards, Jun Lu and Mark Glover

Changes in chromatin, which is the functional state of DNA in a cell, is integral to DNA damage response (DDR). These changes commence from the nucleosome level, which is the fundamental building block of chromatin. At double strand breaks, DNA damage signaling marks the initiation of DDR wherein serine 139 on H2Ax (a variant of H2A) is phosphorylated by Ataxia-Telangiectasia mutated kinase. This signal leads to the concerted recruitment of repair factors. Pioneering work from several labs have shown that this phosphorylated serine 139 is recognized by mediator of cell cycle check point protein (MDC1) via its tandem BRCT domain. This domain is a protein-protein interaction platform and recruits downstream repair factors to facilitate the process. Previously our lab and others have solved the crystal structures of BRCTs with phosphopeptide (pS) mimicking the phosphorylated H2Ax tail. This interaction with nucleosome remained to be deciphered. In this project, we aimed at obtaining structural insights into phosphorylated nucleosome (pS139-H2Ax), in the presence and absence of MDC1 BRCTs. First, we developed effective methods of preparing nucleosomes and phosphorylated nucleosome. Next, we performed electrophoretic mobility shift assay (EMSA) and microscale thermophoresis (MST) to confirm binding of BRCT with phosphorylated nucleosome. Interestingly, the BRCT did not bind to non-phosphorylated nucleosome, indicating a phosphorylation driven binding event. We used cryogenic electron microscopy (cryo-EM) to decipher the structures of modified nucleosomes. The structures (resolution: 3 – 4 Angstrom) reveal interesting inter-nucleosome packing and dynamics, both with and without phosphorylation. Histone tails are flexible and are often not resolved using cryo-EM. We used molecular dynamics to decipher the dynamics of histone tails including that with MDC1 BRCT bound to pS139-H2Ax. This study, using combinatorial techniques of cryo-EM and molecular dynamics, reveals the structure and dynamics in post translationally modified nucleosomes in the presence and absence of MDC1 BRCTs.

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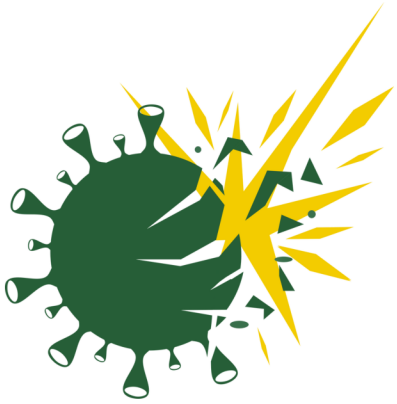

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