ORAL ABSTRACTS

KSHV infection of oral epithelial cells leads to rapid degradation of FOXK1/2, key mediators of the host antiviral response

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic virus that replicates in oral epithelial cells and is transmitted primarily through the oral route via viral shed in saliva. Understanding the key host-pathogen events supporting lytic infection could lead to the development of targeted therapies. We demonstrated that KSHV infection of telomerase immortalized gingival keratinocytes (TIGK) leads to the rapid transcriptional induction of the Forkhead transcription factor FOXQ1, and that the presence of FOXQ1 sustains the lytic cycle. However, the role of other Forkhead factors during infection of oral epithelial cells is still largely unknown. Another Forkhead factor, FOXK1, was recently shown to promote host antiviral responses through transcriptional activation of STAT1, STAT2, and Type I interferon-stimulated genes. Since FOXK subfamily members FOXK1 and FOXK2 are both expressed in TIGK cells, we hypothesized that the FOXK family may play a key antiviral role in oral epithelial cells. We found that siRNA-mediated depletion of FOXK1/2 prior to KSHV infection of TIGK cells enhanced lytic viral gene expression at 3 days post-infection. Notably, FOXK2 depletion in TIGKs decreased the expression of STAT1/2 as well as STAT1/2 downstream targets, suggesting that FOXK2 maintains antiviral responses in the oral epithelium. Furthermore, we found that KSHV infection of TIGK cells lead to the rapid decrease of FOXK1/2 protein levels by 1-hour post infection, while mRNA levels remained unchanged. Treatment of KSHV-infected TIGK cells with proteasomal inhibitor MG132 stabilized FOXK1/2 proteins, suggesting a virus-triggered protein degradation event. FOXK1/2 were also degraded following primary infection of lymphatic endothelial cells and during lytic reactivation of primary effusion lymphoma cells in a manner independent of KSHV replication. Overall, we unveil an antiviral role for FOXKs as suppressors of lytic infection in oral epithelial cells and find that KSHV has evolved mechanisms to rapidly degrade FOXK1/2 proteins to promote its lytic cycle.

UF Virology Retreat 2022

Abstract Submission

Presenter: Dr. Nicole Lynn-Bell (Trainee)

Insects are highly diversified animals that provide many ecosystem services but also threaten agricultural systems. It is increasingly recognized that insects are routinely infected with heritable symbionts, which are those passed from mother to offspring, capable of mediating key ecological interactions. For example, the pea aphid, *Acyrthosiphon pisum*, a pest of forage legumes including alfalfa, is infected with one or more of eight symbionts. Most have one or more heritable facultative symbionts (HFS) that protect against biotic and abiotic threats. Among the best-studied aphid HFS is *Hamiltonella defensa*, which confers resistance to parasitic wasps, but only if the bacterial symbiont is itself infected with a toxin-encoding bacteriophage (virus) called APSE. To add to this complexity, levels of anti-parasitoid protection vary depending on *H. defensa* strain and associated APSE variant. *H. defensa* strains were found to be surprisingly diversified by APSE bacteriophages that also determined the degree of host protection against a natural enemy. Additionally, when phages were horizontally transferred to new phage-free hosts, they rapidly equipped the host to express a resistance phenotype. This is a great example how viruses can have cryptic roles (e.g. spreading resistance genes) that drive host population evolution. Given that infection with APSEs underlie aphid resistance to parasitoids, it is important to understand their genetic diversity as successful biological control relies on understanding the types and frequencies of resistance in target populations.

Using Citrus tristeza virus (CTV)-based vector as a platform for the management of Huanglongbing (HLB).

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Citrus is commercially propagated from elite scion lines lacking tolerance to HLB on selected rootstocks. *Candidatus liberibacter asiaticus* (Clas), the causative agent of HLB, is transmitted between trees by the Asian citrus psyllid (ACP) insect vector. Clas and CTV colocalize in the phloem tissue of citrus where ACP feeds. To induce resistance/tolerance to HLB, systemic but nonvirulent CTV-T36 based vectors are being used to deliver potential therapeutics to the citrus phloem tissue. First, a major therapeutic to manage HLB is antimicrobial peptides (AMPs). CTV delivered AMPs are screened in both the greenhouse and in the Florida orchards after acquiring the required permits. A second use of the CTV vector is to identify CRISPR targets by inducing RNA interference (RNAi) against negative regulators of citrus defense and susceptibility genes. A third use of the CTV vector is to modify psyllid citrus phloem diet by RNAi and bacterial pesticidal proteins. Efficacious CTV-delivered therapeutics will be used in budwood sources as a remedy until a permanent solution for HLB is available. Furthermore, CTV vectors have other uses that include promoting early flowering by overexpressing genes promoting flowering and silencing negative regulators of flowering.

Isolation of novel bacteriophages against a library of clinical antibiotic-resistant isolates of *Pseudomonas aeruginosa: A cell-based approach to improved phage therapy*

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Every year, an estimated 35,000 individuals in the United States are killed by antibiotic-resistant bacteria. This number is expected to increase tenfold by 2050 if no effective alternative to antibiotic therapy is found. Bacteriophages (phages), viruses that target and infect bacteria, provide a promising tool for the treatment of antibiotic-resistant bacterial infections. Despite recent advancements, selecting phages that exhibit specificity for *Pseudomonas aeruginosa* (*P. aeruginosa*)—a multidrug-resistant, opportunistic pathogen that commonly infects ill, hospitalized patients—has been challenging mainly because of the knowledge gaps in understanding virus-host interactions. First, we isolated and characterized eight unique bacteriophages that target over 90% (51/55) of clinical isolates of *Pseudomonas aeruginosa*, each with a unique antibiotic resistance profile and a sequenced genome. Second, we tested our isolated phages in combination with antibiotics and found potential synergistic effects. Finally, utilizing a knockdown library of all non-essential *P. aeruginosa* genes, we are beginning to reveal bacterial genes that are involved in phage infection. Knockout mutations of these genes lead to a significant increase or decrease of bacterial sensitivity to the invading virus. Recognized as one of the largest threats to global healthcare, antibacterial resistance continues to press the need for alternative therapies. Our bacteriophages have shown strong specificity towards diverse clinical isolates of *P. aeruginosa* and have shown increased efficacy in combination with antibiotics. Furthermore, the discovery of genes vital for bacteriophage infection will reveal a new mechanistic understanding of phage-bacteria interactions, which will lead to improved therapy.

Title

Deletion of the LAT promoter from two HSV-1 strains results in drastically different phenotypes

Authors

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Abstract

300 words maximum total length

Herpes Simplex Virus 1 (HSV-1) establishes latency in neurons and expresses a long non-coding RNA termed the latency associated transcript (LAT). The LAT has been implicated in several aspects of viral infection, including the regulation of latency. Previous studies that examined the LAT's influence on histone modifications on viral genomes during latency using HSV-1 recombinants with a 202 bp deletion in the LAT promoter resulted in opposing findings. The Bloom lab found increased levels of the heterochromatin mark H3K27me3 on latent viral genomes from latently infected murine dorsal root ganglia, whereas the Knipe lab observed a decrease in H3K27me3 in murine trigeminal ganglia. In addition, these studies used two different viral strains (17*syn*+ vs KOS, respectively) and mice were infected with different doses as well. Recent work by our lab has shown that 17*syn*+ and KOS display distinct differences in heterochromatin levels during latency. This raised the question whether function or regulation of the LAT differs between these strains and if this could account for the previous discrepancies seen with the LAT promoter deletions. To determine whether there are strain specific differences in the role of the LAT, we examined two recombinants with 202 bp LAT promoter deletions, 17ΔPst and KOSΔPst, in the human neuronal cell line model of latency and reactivation (LUHMES). We found that the LUHMES recapitulate the observations seen in previous experiments where deletion of the LAT promoter results in an increase in H3K27me3 levels compared to the parental strain 17*syn*+ but a decrease compared to the parental strain KOS. We also found distinct differences in the production of viral transcripts and proteins during latency. These results indicate that the function and/or regulation of the LAT may differ between HSV-1 strains and may help explain discrepancies found in the literature when examining the function of the LAT.

Hypoxia prevents the establishment of latency and promotes lytic *de novo* KSHV infection

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Latent infection by Kaposi's sarcoma-associated herpesvirus (KSHV) is a prerequisite for both persistent infection and development of KSHV-associated cancers. Previous studies showed that hypoxia is a natural stimulus of reactivation of KSHV from latency and plays an important role in viral pathogenesis. However, the effect of hypoxia on KSHV primary infection has not been investigated. The goal of this study was to investigate how hypoxia influences the outcome of primary KSHV infection. To test this, we infected cells with KSHV under normoxia (control) and hypoxia, then performed a comparative analysis of viral gene expression and chromatinization of the KSHV genome after infection. To establish the hypoxia condition, we treated the cells with the hypoxia mimetic agent $CoCl_2$ or the cells were cultured under 1% O_2 during infection. We found that hypoxia strongly induces viral lytic gene expression and viral replication following infection in cells in which the virus normally establishes latency under normoxia. Our ChIP analysis revealed that hypoxia reduces the level of repressive histone marks and increases the enrichment of activation histone marks on lytic viral promoters thereby promoting the formation of a transcriptionally permissive chromatin on the KSHV genome. We also found that the overexpression of Hypoxia-Inducing Factor 1α (HIF-1α) under normoxia induces viral gene expression during *de novo* KSHV infection while the siRNA inhibition of HIF-1α during hypoxia abrogates lytic *de novo* KSHV infection. Furthermore, our results show that KSHV lytic proteins further stabilizes HIF-1α protein expression therefore forming a positive feedback loop, which facilitates lytic KSHV infection under hypoxia. Altogether, our data indicate that the expression of hypoxia-induced HIF-1α is crucial for lytic KSHV infection and prevent the establishment of latency following primary infection.

Micropatterning method unraveled distinct cell behavior at intestinal wounds during virus infection

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The intestinal epithelium separates the human body from the outside environment. An intact intestinal epithelial cell (IEC) monolayer is essential to maintain barrier function and restricts viruses to the lumen side, avoiding pathogen trespassing and systemic infection. However, normal peristalsis of the intestine and diseases can lead to damage of the intestinal monolayer creating a wound in the tissue. Interestingly, we observed that IECs localized at wounds support significantly stronger enteric virus infection as compared to cells embedded in the intact epithelium suggesting that wound cells have a different molecular program than internal cells. To control the location, size and number of wounds within an epithelium, we recently developed a micropatterning method that allows us to grow IECs in standardized populations. This method allows each cell to have a defined local density and localization where the edge of each population represents a wound and the center represents the intact epithelium.

Using this bioengineering method, we were able to confirm that intestinal cells at a wound are significantly more infected by viruses than center cells. Surprisingly, viruses were endocytosed by center cells, but could not efficiently replicate. Additionally, we found that cell localization within the population (edge vs center) and local density determined cell behavior. For instance, edge cells or cells characterized by low local density used other endocytic pathways as compared to center cells at high local density. Also, the antiviral interferon (IFN)-dependent immune response showed distinct patterns, in which center cells elicited a higher basal immune activation as compared to edge cells. All together these results point to novel ways that IECs control their response to virus infection and suggest that this unique response is critical for gut homeostasis.

The role of a host bile acid transporter in norovirus pathogenesis.

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Norovirus is the leading cause of severe childhood diarrhea around the world and a major cause of acute gastroenteritis in all age groups. Despite the health burden, very little is known about the pathogenic mechanisms underlying gastroenteritis symptoms. To gain further understanding of this important virus, murine norovirus (MNV) has been used as a model system for many years and has led to significant advances in understanding norovirus biology. We recently discovered that genetically wild-type neonatal mice develop acute, self-resolving diarrhea when infected with MNV, a disease course that mirrors human norovirus infection. Virulent strains (MNV-1 and WU23) infect intestinal immune cells at the peak of disease whereas attenuated CR6 does not. The mechanisms by which MNV transcytoses the epithelial barrier to reach its immune cell targets during symptomatic infection is unknown and is the goal of my research project. MNV-bile acid interactions have been characterized in other steps of MNV infection, specifically in the binding of the viral entry receptor. Bile acids are recycled from the small intestine back to the liver by the apical sodium bile acid transporter (ASBT). ASBT has been shown to be able to not only bind individual bile acids but bile acid complexes as well causing endocytosis of the ASBT-bile acid complex and shuttling of the complex through the enterocyte to the basal surface. Specifically, I will test the hypothesis that MNV complexes with bile-acid in the small intestine upon infection. The MNV-bile acid complexes bind ASBT in the distal small intestine, and the ASBT-MNV-bile acid complex is endocytosed into the enterocyte. ASBT is recycled back to the cell surface while the MNV-bile acid complex moves to the basal surface for release. Preliminary data shows that *Asbt*$^{-/-}$ neonatal mice develop significantly less disease upon MNV infection compared to WT mice.

Title: Assessment of a mass balance equation for estimating community-level prevalence of COVID-19 using wastewater-based epidemiology in a mid-sized city

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Abstract

Wastewater-based epidemiology (WBE) has emerged as a valuable epidemiologic tool to detect the presence of pathogens and track disease trends within a community. WBE overcomes some limitations of traditional clinical disease surveillance as it uses pooled samples from the entire community, irrespective of health-seeking behaviors and symptomatic status of infected individuals. WBE has the potential to estimate the number of infections within a community by using a mass balance equation, however, it has yet to be assessed for accuracy. We hypothesized that the mass balance equation-based approach using measured SARS-CoV-2 wastewater concentrations can generate accurate prevalence estimates of COVID-19 within a community. This study encompassed wastewater sampling over a 53-week period during the COVID-19 pandemic in Gainesville, Florida, to assess the ability of the mass balance equation to generate accurate COVID-19 prevalence estimates. The SARS-CoV-2 wastewater concentration showed a significant linear association (Parameter estimate = 39.43, P-value<0.0001) with clinically reported COVID-19 cases. Overall, the mass balance equation produced accurate COVID-19 prevalence estimates with a median absolute error of 1.28%, as compared to the clinical reference group. Therefore, the mass balance equation applied to WBE is an effective tool for generating accurate community-level prevalence estimates of COVID-19 to improve community surveillance.

Evidence of a Sjögren's disease-like phenotype following COVID-19

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Abstract

Sjögren's syndrome (SS) is a chronic and systemic autoimmune disease characterized by lymphocytic infiltration and the development of dry eyes and dry mouth as the result of the secretory dysfunction of the lacrimal and salivary glands. In recent years, infectious agents have been shown to be associated with SS, including Cytomegalovirus, Coxsackie, Epstein-Barr virus (EBV), and lymphotropic virus-1 (HTLV-1). Studies suggest that infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may trigger an autoimmune response, as evidenced by increased autoantibodies in patients diagnosed with Coronavirus disease 2019 (COVID-19). To investigate the relationship between SARS-CoV-2 infection and SS, this study used SARS-CoV-2 to infect humanized Angiotensin-converting enzyme 2 (ACE2) mice. Mice infected with the virus showed dry eye symptoms, decreased saliva flow rate, elevated antinuclear antibodies (ANAs) and anti-SSB/La, and lymphocyte infiltration in the lacrimal and salivary glands. We detected the viral nucleocapsid protein in mice exocrine glands with significant apoptotic bodies by the acinar cells. Confirmed with clinical data, we also observed the elevation of SS-specific autoantibodies (ANA, anti-SSB/Ro52, and anti-SSA/La) and specific ANA patterns in sera from COVID-19 patients. One unique aspect of SS is the high degree of sexual dimorphism, with women being affected 10-20 times more than men. To determine whether COVID-19 patients exhibited sexual dimorphism in the autoantibody response, we grouped the sera by sex. We found the male patients showed elevated anti-SSA/Ro52 compared to female patients ($p=0.0029$), and female patients had more diverse ANA patterns. Lastly, monoclonal antibodies isolated from recovered patients using single-cell antibody nanowells technology were shown to recognize the nuclear antigens. Overall, by observing SS-like phenotypes in mouse models and patients, our study confirms a direct pathogenic role of SARS-CoV-2 in SS.

Virus Sequence Discovery in the Small Hive Beetle

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Acknowledgements:
Dr. Bryony Bonning, Dr. Sijun Liu, Dr. Cindy Goodman, Dr. Adam Dolezal, Dr. Ruchir Mishra. This project was supported by the USDA National Institute of Food and Agriculture, award number 2021-67013-33559.

The small hive beetle (SHB), *Aethina tumida*, causes significant damage to apiaries. We aimed to characterize the virome of SHB to 1) address the potential role of SHB in honey bee virus transmission, and 2) identify SHB viruses with potential for use in biological control of this pest. Insects from multiple regions in the U.S. were either field caught or lab-reared prior to freezing. RNA was extracted and sequenced, and the transcriptome assembled using Trinity. Contigs were annotated by BLASTx. Sequences from multiple honey bee viruses were found in field-caught SHB but not in lab-maintained insects. This result suggests that honey bee viruses were eliminated from beetles maintained in the lab, supporting the hypothesis that honey bee viruses do not replicate in SHB. We will test this hypothesis by infecting SHB cell lines with these viruses and monitoring viral replication. The near-complete sequence of a novel, putative phasmavirus (order: *Bunyavirales*) was discovered from field-caught adult beetles. In addition, a partial glycoprotein sequence from a different phasmavirus was integrated into the genome of SHB in all samples. We plan to sequence the small RNAs of SHB to determine whether there is active replication of the putative phasmavirus. Whether the putative SHB phasmavirus has potential for biological control of SHB remains to be determined.

POSTER ABSTRACTS

Poster 1

EBV LMP-1 FORMS A K^+ SELECTIVE ONCOCHANNEL THAT SUPPORTS LYTIC VIRUS RELEASE

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Viruses manipulate ion membrane potentials to facilitate their replication, pathogenicity, and persistence. With K^+ efflux a known trigger of inflammasome activation, and the inflammasome recently linked to EBV lytic activation, we asked if EBV regulates plasma membrane K^+ potential during its lytic phase. Using a flow cytometric assay to measure K^+/Tl^+ transport, we find that K^+ transport increases as EBV progresses through its lytic phase. Blocking K^+ efflux further prevents virus production and release without inhibiting viral DNA replication. A search for hidden Markov model similarities to known K^+ channels revealed similarity between EBV Latent membrane protein-1 (LMP-1) and the HIV-1 Viral protein U, a known cationic channel.

While LMP-1 is considered to be the most potent EBV oncoprotein during latency, little is known about LMP-1's intrinsic properties or if/how it contributes to EBV lytic replication. We find that lytic induction leads to full length LMP-1 expression and its progressive localization to the plasma membrane over the course of the lytic phase. De novo modeling predicts a circular arrangement of the LMP-1 transmembrane spans, and electrophysiology of epithelial cells expressing LMP-1 reveals a high flicker type K^+ channel activity at negative voltages with a single-channel conductance of 105 +/-8 pS. To determine if the K^+ channel function is intrinsic to LMP-1 or indirect (via signaling), we removed all of the known cell signaling components leaving only the transmembrane spans intact. This opened the channel at negative *and* positive potentials as well as increased conductance to 250 pS revealing this to be an intrinsic function. To our knowledge, LMP-1 represents the first viral oncochannel – and a novel target for channel-blocking antiviral/anticancer agents.

Poster 2

Thermal and pH tolerance of *Vibrio parahaemolyticus* bacteriophages

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Vibrio parahaemolyticus (Vp) is a bacterial pathogen that occurs naturally in coastal waters and can be transmitted to humans through the consumption of raw or undercooked seafood, most notably oysters. Control of *V. parahaemolyticus* is complicated due to the growing issue of antimicrobial resistance and by limits on the use of antibiotics in food production processes. Bacteriophages, viruses that infect bacteria, represent a potential alternative solution to this issue. We sought to characterize the environmental persistence of two previously isolated wild-type *V. parahaemolyticus* bacteriophages — Phage 9 (P9) and Phage 10 (P10). To analyze pH tolerance of P9 and P10 we incubated the virus in pH buffer solutions for one hour before performing overlay plaque assays. Under these conditions, both bacteriophages remained stable for pH values 4 through 10 with an average titer of 5 log PFU/mL and 6 log PFU/mL, respectively, and with P10 also remaining stable at pH 11 ($p>0.05$). Activity of P9 was reduced to 4.5 log PFU/mL at pH 11 ($p<0.05$) and both phages reduced their infectivity to below the 1.0 log PFU/mL limit of detection at pH extremes of 3 and 12 ($p<0.05$). Thermal tolerance was tested by exposing the bacteriophages to 20-80ºC for one hour. P9 remained stable at 20-50°C (6 log PFU/mL), then reduced by a 2-log at 60°C and to below the 1-log PFU/mL limit of detection at 65°C ($p<0.05$). P10 had an average titer of 6 log PFU/mL at 20-40°C and reduced by 1 log PFU/mL at 50°C ($p<0.05$), with no significant reduction from 50-60°C ($p>0.05$). The titer of P10 was reduced by 2 log PFU at 70°C and was totally inactivated at 80°C ($p<0.05$). Both bacteriophages showed promising stability over pH and thermal treatment. Our future research efforts are focused on determining the lytic activities of these bacteriophages. Our findings contribute to validation of these bacteriophages as a biocontrol for *Vibrio parahaemolyticus* in seafood.

Poster 3

Identification of Israeli Acute Paralysis Virus receptors in the gut of the honey bee

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Colonies of the western honey bee, *Apis mellifera,* have been severely impacted in recent years by a wide range of stressors. The causes of bee declines are multifactorial, but *Varroa* mites and associated viruses are among the most serious threats to honey bee health. The dicistrovirus, *Israeli acute paralysis virus* (IAPV) is of particular concern in relation to colony losses.

IAPV is hypothesized to invade midgut epithelial cells of honey bees by receptor-mediated endocytosis, but the specific molecular mechanisms of this process have not been revealed. By feeding adult honey bees on a phage display library, peptides that bind the honey bee midgut were identified. The most enriched peptide, Bee midgut Binding Peptide (BBP2.1), shared 86% identity with a region of the IAPV capsid protein. This region of the capsid protein is likely to be instrumental in virus interaction with the honey bee gut receptor. BBP2.1 was cloned and fused with mCherry for further analysis. Peptide binding to honey bee gut brush border membrane vesicles (BBMV; enriched in membrane proteins) was confirmed *in vitro* by pull-down assay with all the peptide-mCherry fusion protein binding to BBMV. Competition assays showed that the peptide competes with IAPV virions for binding to honey bee gut-derived BBMV suggesting that the peptide and the virus bind to the same protein or proteins. UV-cross-linking of BPP2.1 and associated BBMV proteins resulted in the identification of five candidate binding partners on the surface of the honey bee gut epithelium. Two of these proteins have been expressed in insect cells using the baculovirus expression system to assess their ability to mediate cell entry of IAPV coat proteins and virions. Results will be discussed in the context of practical applications such as the use of virus-blocking peptides toward suppression of virus infection.

Poster 4

Evaluating The Transmission-Blocking Properties of Niclosamide Against Zika Virus

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Zika virus (ZIKV) is transmitted between humans by the bite of an infected female *Aedes aegypti* mosquito. While there have been no confirmed local cases in the continental United States in 2022, there were large outbreaks in 2016-2017 with over 37,000 cases locally acquired in the US states and territories. There is no vaccine or approved treatment available to prevent transmission meaning another outbreak remains possible. We explored the utility of a World Health Organization essential medicine, niclosamide (NIC) to block ZIKV transmission to mosquitoes. The drug has been shown to inhibit SARS-CoV-2 replication and acts as a broad-spectrum inhibitor against flaviviruses in mammalian tissue culture, which makes it an ideal candidate to test its transmission-blocking potential against ZIKV. However, the antiviral activity of NIC in insect cells has not been evaluated. We hypothesized that adding NIC to an infectious blood meal, in safe concentrations that would be found in the blood of treated humans, will block ZIKV from infecting mosquitoes. We tested whether non-toxic mammalian blood concentrations of 0.003 to 18 µM would be equivalently non-toxic in the *Ae. aegypti* cell line, Aag2 C3, then used dosages of NIC we found to be non-toxic (0.003 to 1.0 µM) to test inhibition of ZIKV in cells. We determined infection prevalence by immunofluorescence assay (IFA) and replication rate by reverse transcription quantitative polymerase chain reaction (rt-qPCR). We then infected *Ae. aegypti* mosquitoes with blood meals containing ZIKV (5 x 10^6 PFU/mL) and the indicated concentrations of NIC (0.3 to 10 µM) and used the same methods to assess NIC inhibition of ZIKV replication in the mosquito midgut. Contrary to results in mammalian cells, NIC does not inhibit ZIKV replication in mosquito cells suggesting a different mechanism of action of NIC or a difference in the viral replication cycle between the mosquito and mammalian hosts.

Poster 5

Title

The role of the ubiquitin ligase Cul4b in B lymphocytes

Authors

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Abstract

Vaccination or infection induces secretion of antibodies by long-lived plasma cells, providing powerful and durable immune protection against secondary infections. Long-lived plasma cells originate from B lymphocytes that have undergone affinity maturation, which requires somatic hypermutation in rapidly proliferating B lymphocytes, creating single strand DNA breaks that must be repaired by the mismatch repair machinery. Cullin 4b (Cul4b) is an E3 ubiquitin ligase found in most cells, including B lymphocytes, but its function in B lymphocytes is unknown. Cul4b serves as one of two docking proteins that form the backbone of the Cullin RING Ligase 4 (CRL4) complex, which ubiquitinates substrate proteins and drives their proteasomal degradation. Previous studies in cell lines have demonstrated that Cul4b helps coordinate DNA repair with cell proliferation, and high Cul4b is associated with aggressive disease in colon cancer. Because proliferation and DNA repair are essential for humoral immunity, we hypothesized that Cul4b would also function in B lymphocytes. To test this, we generated mice that lack Cul4b only in B lymphocytes. We immunized these mice with an mRNA vaccine against an H1N1 Influenza A virus, and measured virus-specific serum antibody. We determined that Influenza-specific antibodies were dramatically reduced in the serum of mice that lacked Cul4b in B lymphocytes. To determine if Cul4b promotes DNA damage repair in B lymphocytes, we induced single-strand DNA breaks with UV irradiation and then stimulated the cells to proliferate in vitro. We found that Cul4b promoted B lymphocyte proliferation after UV exposure. Understanding the Cul4b-DNA damage repair pathway in B lymphocytes will identify new drug targets to transiently amplify this pathway to improve vaccines and encourage stronger antibody responses, or to inhibit this pathway to prevent tumor progression in the context of cancer.

Poster 6

Preliminary characterization of a new rodent paramyxovirus

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The family *Paramyxoviridae* is comprised of enveloped RNA viruses that infect mammals, birds, reptiles and fish. There are several paramyxoviruses that affect humans, including measles -, mumps -, and parainfluenza viruses. Paramyxoviruses pose particular risks to both humans and animals as they are frequently associated with respiratory infections and inter-species transmissions. It is important to identify and characterize these viruses because of their potential to jump species and cause serious infections in humans, as exemplified by Hendra and Nipah viruses. A novel paramyxovirus was opportunistically isolated from the kidneys and spleen of a dead rodent found in Gainesville, and its genome determined through next-generation sequencing. Preliminary phylogenetic analyses established the paramyxovirus group to which the novel virus likely belongs to. Fifteen different cell lines including bat, canine, deer, human, non-human primate and rodent cells, were inoculated to examine cell and host tropism. Avicel cellulose plaque assays were performed on supernatant samples obtained six days post-inoculation to determine the viral titer produced in each cell line, and we developed an RT-PCR test to detect the viral RNA. Preliminary results indicate that this novel paramyxovirus can complete its life cycle in human, non-human primate, and rodent cells, suggesting that the virus may be able to infect humans. We next wish to perform serology tests to explore whether Gainesville, Florida, residents have been exposed to this virus.

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Poster 7

Title: Antibody Response to Mosquito Salivary Proteins as a Marker for Exposure

Authors: Veronique Etienne, MS, Adriana Gallagher, Maureen Long, DVM, PhD, DACVIM-LA, Derek Cummings, PhD, MHS, MS

Arboviruses (arthropod-borne viruses) are responsible for a massive global burden of disease in humans. *Aedes aegypti* and *Aedes albopictus* mosquitoes spread the majority of human mosquito-borne diseases (MBDs). Mosquito population surveillance is key to assessing mosquito/vector population and determining appropriate interventions when MBD outbreaks occur. However, traditional methods are cumbersome and costly; therefore, there is a need for more efficient mosquito surveillance. The quantitative measuring exposure to mosquito bites (and possible arbovirus exposure), via technologies such as ELISA, has been demonstrated as a promising alternative to trap-based surveillance. Using PRISMA guidelines, a systematic review and pooled analysis were performed to assess the efficacy of detection of human antibody (Ab) response to mosquito salivary proteins (MSP) as presented in the literature. A total of 1353 studies were screened by two reviewers; 103 articles were included in the qualitative synthesis. The pooled analysis included 23 papers met our inclusion criteria, provided individual level human IgG response to MSP via ELISA. We assessed how subject age, *Aedes* spp. mosquito, antigen type, collection season, population level of mosquito exposure, and Koppen-Geiger climate impact OD values in separate univariate analyses as well as a multivariate analysis. We found that OD values correlated positively with antigen complexity as well as population level of mosquito exposure. While there is considerable variation between studies (ICC=0.12), using human IgG holds promise in complimenting more traditional mosquito surveillance methods as a proxy for individual and population exposure to *Aedes* spp. mosquitoes.

Poster 8

Favipiravir Suppresses Zika Virus Replication Through Activity as a Mutagen

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This study aims to evaluate the antiviral effect of the nucleoside analogue favipiravir (FAV) against Zika virus (ZIKV) in three human derived cell lines. HeLa (cervical), SK-N-MC (neuronal) and HUH-7 (liver) cells were infected with ZIKV and treated with different concentrations of FAV. Viral supernatant was sampled daily and infectious viral burden was quantified by plaque assay. Changes in viral infectivity were quantified by calculating specific infectivity, and FAV effect on viability of infected and uninfected cells was assessed in each cell line. FAV activity was most pronounced in HeLa cells, as treatment caused marked declines in infectious titers and viral infectivity that occurred in a concentration dependent manner and became more pronounced at increasing FAV exposure times. Additionally, toxicity studies showed that FAV substantially improved viability of infected HeLa cells. Although SK-N-MC and HUH-7 cells were susceptible to FAV's anti-ZIKV activity, similar effects on viral infectivity and improvements in cell viability were not observed. Next, we investigated FAV's mechanism of action against ZIKV to explain tissue dependent disparities in drug effect. These results showed that therapy induced a bias toward transition mutations and promoted production of defective viral particles in all three cell lines however, defective viral particles made up a larger portion of the viral population released from HeLa cells both at increasing FAV concentrations and exposure times. Quantification of intracellular drug concentrations revealed that intracellular levels of FAV and its active metabolite, FAV-RTP, were also highest in HeLa cells. FAV's ready uptake and activation in HeLa cells likely facilitated its incorporation into viral genomes and contributed to an increased production of defective viral particles, thus causing enhanced activity in this cell line. These studies revealed FAV's mechanism of action against ZIKV and highlight the host cell's impact on the activation and antiviral activity of nucleoside analogues.

Poster 9

A Biophysical Study of AAV Genome Ejection

Keely Gliwa[1],Antonette Bennett[1], Austin Kansol[1], Joshua Hull[1], Courtnee Edington[1], Renuk Lakshmanan[1], Tyler Schult[1], Paula Barreto[1], Paul Chipman[1], Hazel Levy[1], Mavis Agbandje-McKenna[1], Robert McKenna[1]

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Adeno-associated Virus (AAV) are the most widely studied viral vector for clinical gene therapy applications. Consequently, a significant amount of research has focused on understanding all aspects of the virus life cycle. An AAV infection is initiated by receptor/co-receptor binding, clathrin coated-mediated endocytosis, endo-lysosomal (pH 7.4 - 4.0) transport and escape to the cytoplasm, nuclear localization, and entry into the nucleus via the nuclear pore, where genome ejection occurs in the nucleus at pH 7.4. Genome ejection is therefore the final critical step for replicating and expressing the therapeutic gene. In this study, we present the development of a thermal assay to probe the DNA ejection process using two structurally diverse AAV serotypes, namely AAV2 and 5. Previously, differential scanning fluorometry studies determined the thermal melt temperatures (Tm) of AAV2 and 5 in PBS as 68 and 89°C, respectively. We have shown, using this thermal assay, that both AAV2 and 5 lose ~50% of their packaged genome at ~55°C, in PBS. This data is confirmed by cryo-EM micrographs of the virus at these temperatures. Examination of negative stain EMs reveal conformational changes in the AAV capsid at temperatures > 55°C , ~5°C, before the capsid Tm, when all the capsids are stain penetrated (empties) with ‘strings’ of exposed DNA exiting/ejecting from the capsid. This data would imply that the trigger for genome ejection is independent of the AAV serotype, but the rate required for all genomes to be released is dependent on the thermal stability of the capsid. These thermal assays can therefore be used to screen conditions experienced by the capsid throughout the viral life cycle. We can then measure those effects on genome ejection and ultimately assess how the rate of genome ejection affects the rate of therapeutic gene expression.

Poster 10

Physiologic hypoxia in the human gut influences the immune functions of intestinal epithelial cells

Sorin O. Jacobs, Stephanie Münchau, Megan Stanifer, Steeve Boulant.

The intestinal epithelium serves as a primary barrier toward the external environment. In order to maintain homeostasis, intestinal epithelial cells (IECs) must tolerate commensal microbiota, while remaining immune reactive to enteric pathogens, such as viruses (e.g. reovirus). The intestinal epithelium forms a tight monolayer, which differentiates along a three-dimensional crypt-villi structure. Within this structure, IECs are exposed to a steep oxygen gradient, with normoxic levels of oxygen in the highly vascularized crypt, and low oxygen at the tips of the villi that are most exposed to the hypoxic intestinal lumen, a state called physiologic hypoxia. Physiological hypoxia in the GI tract is known to be essential for commensal microbiota and some inflammatory regulation, but the effect of hypoxia on the innate immune response generated by IECs upon enteric infection remains poorly characterized. Here we have evaluated the effect of hypoxia on interferon production and have shown that hypoxia results in impaired IFN production, resulting in a reduced immune response. This decrease has been traced to decreased and delayed signal transduction, specifically less efficient phosphorylation and nuclear translocation of the transcription factor IRF3, which we can correlate to decreased phosphorylation of the master regulator TBK1. The mechanism for this hypoxia-mediated downregulation of TBK1 activation has been mapped to Casein Kinase 2 (CK2), and further investigation has suggested a role for the protein phosphatase PP2A. The aim of my PhD project is to consolidate our preliminary data to identify a mechanism through which hypoxia may regulate interferon responses through CK2 interaction with PP2A or other factors. We propose that the oxygen gradient helps to regulate immune responses, which constitutes a strategy to maintain gut homeostasis, protecting the stem cell niche while allowing tolerance of commensal microbiota in the intestinal lumen.

Poster 11

Gammaherpesvirus-mediated repression of EWSR1 reveals EWSR1 to be a negative regulator of B cell response

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Abstract

The germinal center (GC) plays a central role in the generation of antigen-specific B cells and antibodies. Tight regulation of the GC is essential due to the inherent risks of tumorigenesis and autoimmunity posed by inappropriate GC B cell processes. Gammaherpesviruses such as Epstein–Barr virus (EBV) and murine gammaherpesvirus 68 (MHV68) utilize numerous armaments to drive infected naïve B cells, independent of antigen, through GC reactions to expand the latently infected B cell population and establish a stable latency reservoir. EWSR1 (Ewing sarcoma breakpoint region 1) is a transcription and splicing regulator that is widely recognized for its involvement as a fusion protein in Ewing sarcoma, but that has not previously been identified to play a role in gammaherpesvirus infection or in normal B cell responses. We recently demonstrated that the MHV68 microRNA (miRNA) *mghv-miR-M1-7-5p* represses host EWSR1 to promote expansion of MHV68 latently infected GC B cells. In work presented here, we further demonstrate that EWSR1 regulated normal GC B cell responses to a nonviral antigen, outside of the context of infection. EWSR1 deficiency did not affect the proliferation or survival of GC B cells, but instead resulted in the generation of increased numbers of precursor GC B cells in response to a nonviral antigen. Likewise, repression of EWSR1 by *mghv-miR-M1-7-5p* resulted in the generation of increased numbers of precursor GC B cells. Together, these results identify a role for EWSR1 as a negative regulator of both antigen-specific and virus-driven pre-GC B cells and GC B cell responses.

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Poster 12

Elimination of norovirus using commonly used sanitizers of pre-harvest agricultural water

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Human norovirus is the leading cause of foodborne gastrointestinal infections in the U.S. and has been responsible for several outbreaks associated with the consumption of raw produce. The introduction of such microbial food safety hazards onto crops via agricultural water is of significant concern to crop growers. There are abundant water treatment options to combat potential pathogens at pre-harvest levels; however, there is a knowledge gap in the efficacy validation of anti-noroviral sanitizers. This study aimed to validate the treatment efficacy of commonly used commercial irrigation water sanitizers against human norovirus. Tulane virus (TuV), a surrogate for human norovirus, was exposed at 5.4 $\log_{10}$ PFU/ml to two sanitizers in deionized water: SaniDate® 12.0 (120,000 ppm peroxyacetic acid (PAA)/185,000 ppm H_2O_2) and Accu-Tab® (calcium hypochlorite tablet). Exposure was conducted for 5 minutes at 32ºC with incrementally increasing concentrations of sanitizer for each trial, beginning at the minimum recommended concentrations of 4-10 ppm of PAA for SaniDate® and 6-10 ppm of free chlorine (FC) for Accu-Tab®. The antiviral efficacy of each sanitizer against TuV was then assessed with the infectivity (plaque) assay using an LLC-MK2 monolayer. Both products exceeded a 1-$\log_{10}$ reduction at 10 ppm of PAA/FC ($p<0.05$). Pronounced antiviral activity was observed in SaniDate® at 50 ppm of PAA with a 3.0 ± 0.1 $\log_{10}$ reduction, reaching 0.35 $\log_{10}$ PFU/ml limit of detection at 200 ppm ($p<0.05$). Accu-Tab® inactivated TuV to below the 0.35 $\log_{10}$ PFU/ml limit of detection at 50 ppm of FC ($p<0.05$). Future research efforts are focused on testing the anti-noroviral efficacy of sanitizers using actual agricultural water samples collected from Florida farms.

Poster 13

Title: The role of host factors EDC4 and XRN1 in promoting human coronavirus replication

Authors: Meera Nair, Marco Grodzki, and Stephanie M. Karst

The COVID-19 pandemic caused by SARS-CoV-2 has resulted in the highest number of infections and deaths caused by a human coronavirus worldwide. Little is known about the host factors required by SARS-CoV-2 to replicate. The Karst lab and colleagues recently performed a genome-wide CRISPR knockout screen for SARS-CoV-2 and endemic OC43 human coronavirus strain to identify proviral host factors, revealing two candidates; EDC4 and XRN1. These factors are involved in the host mRNA degradation pathway. We confirmed a role for EDC4 and XRN1 in coronavirus replication by making targeted knockout (KO) cells and showing that they support reduced viral replication. Our initial hypothesis about the role of EDC4 and XRN1 in the human coronavirus life cycle was that these factors are necessary for viral non-structural protein 1 (NSP1)-mediated host shutoff. We transfected NSP1 and GFP plasmids into wild-type (WT) and KO cells and analyzed NSP1-mediated GFP degradation by immunofluorescence. However, we did not see any difference in GFP degradation between WT and KO cells which suggests that EDC4/XRN1 are not involved in NSP1-mediated host shutoff. The second hypothesis was that EDC4 and/or XRN1 are involved in the formation of viral replication and transcription complexes on the endoplasmic reticulum membranes. If correct, upstream processes like synthesis of viral NSPs should not be affected in KO cells while downstream synthesis of structural proteins should be affected. We found evidence that disputes this hypothesis because upstream NSP synthesis was reduced in EDC4-KO and XRN1-KO cells. Based on this finding, we are testing the alternative hypothesis that EDC4 and XRN1 are involved in the very early steps of entry, genome uncoating or translation of viral RNA.

Poster 14

Geographical partitioning of Mosquito-Associated Viruses from *Aedes aegypti* in Puerto Rico

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Aedes aegypti mosquitoes are the main vectors of dengue, Zika, and chikungunya viruses. Metagenomic sequencing of mosquitoes has revealed novel Mosquito-Associated Viruses (MAVs), some of which, have been shown to modulate vector competence (capacity to transmit pathogens) by enhancing or decreasing replication success of medically important arboviruses. The MAVs are classified into 1) arboviruses (capable of replicating in both vertebrate and mosquito cells), 2) insect-specific viruses (ISV) (only can replicate in insect cells), and 3) viruses with unknown host specificity. A fundamental understanding of *Ae. aegypti* ecology and biology are needed to define how geographical distribution and diversity of mosquito populations determine MAV occurrence and persistence across generations. We hypothesized that ecological and evolutionary factors shape the mosquito core-virome (commonly shared viruses within a specific population), where geographical partitioning into man-made vs. natural habitats drives the diversification of MAVs in *Ae. aegypti* populations, influencing their host biology and their capacity to transmit medically important arboviruses. We are investigating the ecological profile of MAVs in natural *Ae. aegypti* populations from the Caribbean Island of Puerto Rico, where dengue is endemic. To investigate MAV diversity and distribution, we collected samples in 4 geographically segregated populations from 2 urban and 2 rural habitats. Preliminary RNASeq data indicate that the metavirome composition (representing predominantly the Virgaviridae, Totiviridae, and Bunyaviridae) varied between the selected urban and rural habitats. A distinct pattern was observed, wherein urban samples showed a higher variability of MAVs, with the Phasi-Charoen-like phasivirus (PCLV) as the predominant virus found in urban *Ae. aegypti*. This is the first study to conduct a metagenomic analysis in spatially partitioned *Aedes aegypti* populations in Puerto Rico, and we expect that our results will provide a better understanding of how ecological population dynamics can modulate the MAV's profiles within local mosquito populations and potentially influence vector competence.

Poster 15

Quyen Nguyen
Virology Retreat Abstract
10.6.2022

Norovirus is the leading cause of virally induced gastroenteritis across all age groups. Despite the high disease burden, there has yet to be an available vaccine and little is known about the mechanisms noroviruses employ to cause disease. Since the study of human norovirus (HnoV) is limited, the field uses murine norovirus (MNV) to study pathogenesis in the context of a natural host. Pertinent to understanding their pathogenic mechanisms is therefore the tissue and cellular tropism of MNV during symptomatic infection. From asymptomatic adult models of MNV, virulent strains of MNV infect the upper small intestine (SI), the lower SI and disseminate extraintestinally with infection occurring in a variety of immune cells. However, the tissue and cellular tropism during symptomatic infection is unclear. Using our neonatal model of symptomatic MNV infection, we've demonstrated that virulent strains of MNV infect immune cells in the subepithelial space of the small intestine and disseminate extraintestinally to the spleen and liver. I will further delineate the cellular targets of virulent MNV in extraintestinal tissues with the goal to test whether dissemination is a required event for norovirus disease.

Poster 16

Identification of novel host interaction partners of the KSHV tegument protein ORF45

Marley Palmer, Natalie Atyeo, Bernadett Papp
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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic gammaherpesvirus that can replicate in the oral epithelium, promoting viral shedding into saliva, which contributes to viral transmission. The KSHV-encoded ORF45 is an immediate early virion-associated tegument protein, which is vital for efficient lytic reactivation and virus production. Our group discovered that ORF45 is nuclear and can rapidly induce the expression of a lytic cycle promoting host gene during primary infection of oral epithelial cells, a function which is critical for sustained lytic infection of oral epithelial cells. We hypothesize that ORF45 might directly interact with key chromatin regulatory factors, in order to rapidly induce lytic infection promoting genes. Here, we aim to identify novel interaction partners of ORF45, which factors will then be evaluated for their role in lytic infection. Importantly, recent large scale proteomics studies of KSHV factors uncovered chromatin related proteins among the putative ORF45-interaction partners. Following up on these putative interactions, we used coimmunoprecipitation assays to demonstrate that ORF45 interacts with two related host transcription factors (namely FOXK1 and FOXK2) as well as a chromatin regulatory factor called MORF4L2 in epithelial cells and during lytic reactivation of KSHV-infected primary effusion lymphoma cells. As FOXK1/FOXK2 and MORF4L2 are both part of several co-repressor and co-activator multiprotein complexes, we also investigated other subunits of these complexes. We found that ORF45 associates with histone deacetylase 1, 2 and 3 (HDAC1-2-3) as well as MBD3, which proteins are key components of co-repressor complexes, including SIN3A and the Nucleosome Remodeling and Deacetylase complex (NuRD). Importantly, HDAC inhibition is known to promote lytic reactivation and our group recently discovered that depletion of NuRD subunits promotes lytic reactivation. Based on our data, we envision that ORF45 may directly associate with these co-repressor complexes in order to interfere with their function to promote the lytic cycle.

Poster 17

Title: P-body components EDC4 and XRN1 regulate replication of human coronaviruses including SARS-CoV-2

Authors: Marco Grodzki, Andrew Bluhm, Abderrahmane Tagmount, Moritz Schafer, Michael Norris, Christopher Vulpe, and Stephanie M. Karst

As of October 6th, 2022, the COVID-19 pandemic resulted in 620 million cases and 6.5 million deaths globally. Although highly efficacious vaccines are available in most regions of the World, elucidating the molecular mechanisms of replication of SARS-CoV-2 and of other human coronaviruses (HCoVs) is highly important for developing novel antiviral therapies and consequently for a better preparedness to future pandemics.

Host factors required for virus replication represent candidate druggable targets with potential for broad-spectrum activity. In our recent work (Grodzki et al 2022) we performed genome-wide CRISPR knockout screens in hACE2-HEK293T cells transduced with the Brunello library and infected with either SARS-CoV-2 or human coronavirus OC43 strain. We identified previously described host factors that promote human coronavirus replication, as well as 53 novel hits. For mechanistic studies, we have prioritized **EDC4 and XRN1**, host proteins which play key roles in host mRNA decay and localize to P-bodies. Recent published or preprint studies showed that other viruses can hijack P-bodies to build their replication complexes, and HCoVs can induce P-body loss. XRN1 has also been shown to inhibit interferon beta activation, facilitating Influenza A virus replication. Our hypothesis is that EDC4 and XRN1 contribute to the formation of the HCoV replication and transcription complex, and/or they are involved in global host mRNA degradation, promoting infection. We infected physiologically relevant EDC4 and XRN1 knockout hACE2-SAEC cells, showing that replication of both SARS-CoV-2 and OC43 in EDC4 and XRN1-deficient cells is strongly impaired *in vitro*. To assess the importance of EDC4 in SARS-CoV-2 replication *in vivo*, we pretreated mice with Peptide-conjugated morpholino oligos (PPMOs) targeting EDC4, and infected them with SARS-CoV-2, showing reduction of viral titers in their lungs as well as reduced pathological changes. Further characterization of the role of EDC4 and XRN1 in HCoV replication is ongoing.

Poster 18

Joyce Morales

The role of the GPBAR1 (TGR5) receptor in bile acid-mediated immune response against MNV

Noroviruses are the leading cause of gastrointestinal disease worldwide, affecting all age groups but associated with more severe disease outcomes in children, and responsible for over 200,000 deaths annually worldwide. Gut microbiota are known for having many important effects in the host, including metabolic, physiologic, and immunologic effects. Due to the enteric nature of noroviruses, interaction with the gut microbiota is inevitable, and is logical to speculate that they could influence norovirus infection.

Prior studies have revealed that the microbiota exerts a complex and regional interaction on murine norovirus (MNV) infection in adult mice. There is an increasing appreciation that bacterial metabolites, in contrast to bacteria themselves, drive host immune responses that regulate viral infections. In line with this, my mentor's lab revealed that bacterially biotransformed bile acids rescue inhibition of proximal gut MNV infection in microbiota-depleted mice in an interferon (IFN)-dependent manner. Bile acid engagement of receptors including farnesoid X receptor (FXR) and G-protein-coupled bile acid receptor, GPBAR1 (TGR5) can activate antiviral immune responses including type I interferons (IFNs). Consistent with this, pretreatment of mice with a TGR5 agonist, INT-777, significantly reduced MNV titers in adult mice, a phenotype that was dependent on IFN signaling.

While these prior studies in adult mice illuminate opposing roles for microbiota in influencing regional MNV infection, it was unclear what effect they would have on MNV disease outcome because adult mice are asymptomatically infected. Using the neonatal mouse model, in which we can study disease outcome, we have recently demonstrated that microbiota protect neonatal mice from developing severe norovirus diarrhea, highlighting the importance of understanding how intestinal microbes drive antiviral immune responses. Therefore, we hypothesize that microbiota-derived bile acids induce a TGR5-dependent antiviral response that protects from severe norovirus diarrhea.

Poster 19

Inhibitor of DNA Binding Proteins 1 and 2 (ID1 and ID2) Play Opposing Roles during KSHV Infection and Reactivation in a Tissue Specific Manner

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Kaposi's sarcoma-associated herpesvirus (KSHV) is one of two oncogenic human herpesviruses that is primarily transmitted through saliva. During infection, KSHV hijacks the host's molecular machinery in a tissue specific manner to either establish latency, during B cell infection, or lytic replication, during oral infection. The virally encoded Replication and Transcription Activator (RTA) protein is necessary to promote the lytic cycle and is known to possess intrinsic E3 ligase activity. It is poorly understood what host transcription factors (TFs) play a role in promoting the lytic cycle of KSHV during *de novo* infection and which are involved in maintaining viral latency. We have recently shown through RNA-seq that ID2 was rapidly downregulated during lytic reactivation of KSHV in primary effusion lymphoma (PEL) while ID1 expression was induced upon *de novo* infection of primary human gingival epithelial cells. The ID protein family consists of 4 members, ID1 – ID4, which are all dominant-negative regulators of several TFs. While these proteins are well characterized during cell development their role during viral infection is poorly understood. In this study we sought to understand the tissue specific roles of ID1 and ID2 during the biphasic lifecycle of KSHV. During reactivation in B cells, we determined that RTA utilizes N-terminal ubiquitination to degrade ID2 during the lytic cycle. Additionally, we found that ID2 overexpression during reactivation ablates the induction of the lytic cycle of KSHV. In the context of *de novo* infection of telomerase-immortalized gingival keratinocytes we discovered that ID1 is induced and promotes the lytic cycle of KSHV. Taken together our data suggest that the different ID proteins can induce or repress the lytic cycle of KSHV depending on the cellular environment.

Poster 20

VIRAL HIJACKING OF HOST E3 UBIQUITIN LIGASES TO PROMOTE KSHV LYTIC CYCLE

Authors: Kenzie Spires, Eleanor Wind, and Zsolt Toth

Kaposi's sarcoma-associated herpesvirus (KSHV) is a large enveloped, double-stranded DNA virus that is the causative agent of several types of cancer. KSHV causes lifelong infection in humans by establishing latency following primary infections. The viral DNA-binding transcription factor, RTA, acts as the master regulator of the latent-lytic switch and pushes the virus from latency into the lytic cycle. Identifying what cellular and viral proteins RTA is interacting with can help to better understand how RTA promotes the lytic cycle. Using a proteomic approach, we identified the cellular E3 ubiquitin ligase complex RNF20/40 as a novel interacting partner of RTA. One of the major roles of this complex is the monoubiquitination of histone H2B Lysine 120 on cellular chromatin, which is a hallmark of transcriptionally active genes. While the role of RNF20/40 has been studied in the context of host genomes, its potential to regulate viral genes remains largely unexplored. Using co-immunoprecipitation and proximity ligation assays, we showed interaction between RTA and RNF20/40 complex. We also determined that RNF20/40 can greatly and significantly increase RTA-mediated promoter induction. However, this effect was lost when RTA was unable to bind to its target promoters indicating that the binding of RTA to the promoter is necessary for the synergistic induction. Our results also revealed that the inhibition of RNF20 expression impaired viral DNA replication and KSHV production. Overall, our data suggest that RTA is hijacking the cellular E3 ligase complex RNF20/40 to activate viral gene promoters facilitating the progress of the viral lytic cycle.

Poster 21

Role of ANKLE2 during flavivirus infection of *Aedes aegypti* mosquitoes

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The *Flaviviridae* family includes dengue virus (DENV) and Zika virus (ZIKV), both of which are considered significant global health concerns. The main arthropod vector that transmits these viruses in human populations is the *Aedes aegypti* mosquito, which has widespread distribution. The 2016 global outbreak of ZIKV led to the discovery of an association this arbovirus with microcephaly in children. Recently, congenital ZIKV infection in humans was associated with mutations in the ANKLE2 gene, which was previously linked to hereditary microcephaly. Furthermore, the ZIKV NS4a protein interacts with mammalian ANKLE2 and interferes with its functions, resulting in severe microcephaly. ANKLE2 is also present in invertebrates, where mutations in *Drosophila* ANKLE2 results in development of a small brain, and the expression of human ANKLE2 rescues this phenotype. However, nothing is known about the role of ANKLE2 in *Ae. aegypti* mosquitoes that are infected with ZIKV and DENV. We confirmed that ANKLE2 is also expressed in uninfected *Ae. aegypti* cells and mosquitoes. In Aag2-C3 cells infected with ZIKV we observed a reduction in transcript expression at 24 hours post infection (hpi) In contrast, we did not observe any change during the infection with DENV-1, -2 or -4 at 24 and 48 hpi. Current studies are focused on measuring ANKLE2 expression in mosquitoes infected with ZIKV, and on producing murine antibodies to ANKLE2 to understand protein expression and localization in flavivirus-infected or uninfected *Ae. aegypti* mosquitoes. In future experiments we will use RNAi to knock down ANKLE2 transcript in mosquitoes and cells to assess how the loss of ANKLE2 affects viral replication.

Poster 22

The glycolipid GlcCer in the viral envelope facilitates phenuivirus binding to host cells

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During the past decades, highly pathogenic arthropod-borne viruses (arboviruses) have emerged globally, posing a threat to agricultural productivity, life stock, and public health. Yet only few host factors have been functionally investigated for their function during arbovirus entry. Generally, early virus-host cell interactions largely determine the tropism and outcome of the infection. While proteins on the viral surface are commonly used by enveloped viruses to target and enter cells, the role of lipids in the viral envelope during cell entry remains poorly characterized. Investigating the arbovirus Uukuniemi (UUKV), we found that the glycolipid glucosylceramide (GlcCer) is a major component of the viral particle that allows virus binding to host cells. Lipidomic mass spectrometric analysis provided the lipidome of UUKV particles and revealed that GlcCer is enriched in both, infected cells and virions. Pharmacological inhibition and siRNA-mediated silencing of the synthesis of GlcCer in producer cells resulted in viral progeny with reduced infectivity. Consequently, the depletion of GlcCer in virions severely impaired virus binding to target cells and competing ligands for GlcCer prevented virus attachment to different cell types. UUKV belongs to the family of *Phenuiviridae* which includes many pathogenic arboviruses in the order of *Bunyavirales*. When testing the neglected and emerging phenuivirus Toscana virus (TOSV), we confirmed a role of GlcCer also for TOSV binding to target cells. In summary, our results demonstrate that glycolipids can be essential determinants for virus binding to host cells and have strong implications for future studies to identify virus receptors.

Poster 23

Impact of temperature and relative humidity on coronavirus persistence on clamshell food containers

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The COVID-19 outbreak generated a surfeit of hysterics surrounding the transmission and persistence of coronavirus. Studies on person-to-person contact as a vector found this to be a major transmission route. However, our understanding is limited on the secondary route of transmission through contact surfaces. We aimed to examine coronavirus persistence on a polyethylene terephthalate (clamshell) food container surface over six days at different temperatures (4 and 25°C) and relative humidity levels (Rh, 45 and 65%). By inoculating 1.5×1.5 cm plastic container coupons at ~8-$\log_{10}$ PFU per coupon with bacteriophage Phi6, a surrogate for SARS-CoV-2, we performed overlay plaque assays to examine viral infectability over time. We observed a significant reduction in viral titer over time across all conditions. However, when compared to that of 45% at 25°C, 65% at 25°C we saw a greater rate of inactivation over time by 2.5-$\log_{10}$ PFU ($p<0.05$). At Rh of 45%, lowering the temperature to 4°C had marginal impacts on Phi6 viability over the six-day incubation period and resulted in an overall 1.1-$\log_{10}$ PFU reduction; the differences were not always statistically significant across all time periods ($p>0.05$). The data for the condition 4°C (65% Rh) is currently being collected and will allow us to further examine the virus persistence across different temperatures and contact times. Future directions include testing additional contact surfaces and testing the impact that an organic load would have on the viability of Phi6. The outcomes of this study can help food establishments characterize various

Poster 24

HIGH RESOLUTION TRANSCRIPTOME ANALYSIS OF KAPOSI'S SARCOMA ASSOCIATED HERPESVIRUS

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Kaposi's sarcoma (KS) is the most common AIDS-associated cancer caused by KS-associated herpesvirus (KSHV), which is also etiologic for two B cell malignancies. In order to understand the molecular mechanisms underlying KSHV pathogenesis, researchers have made substantial efforts towards functional annotation of KSHV genes. As the conventional annotation of KSHV genome relies on identification of open reading frames (ORFs), we lack information on the complete viral transcriptome for both coding and noncoding RNAs. We have analyzed KSHV transcripts derived from lytically induced (48 hrs) BCBL-1 cells using Transcriptome Resolution through Integration of Multiplatform Data (TRIMD) RNAseq pipeline that integrates data from different sequencing platforms, including Illumina for short-reads, PacBio Isoseq for long-reads and deepCAGE for 5' cap site sequences. Due to the low proportion of KSHV transcripts in total RNA, we enriched for KSHV-specific transcripts in Illumina and PacBio libraries using KSHV specific RNA baits, which significantly increased the percentage of reads aligning to the KSHV genome. Our TRIMD analysis has identified >200 novel 5′ transcription start sites, splice junctions and 3′ poly(A) sites, thus magnifying our current resolution of a high density KSHV transcriptome. We also tested some of the TRIMD identified splice junctions with large introns (>10 Kb) using strand-specific PCR, and were able to successfully validate the presence of these introns. Further, the coding potential analysis of the TRIMD identified transcripts has predicted many novel short/long peptide isoforms of annotated KSHV ORFs. In addition, we identified a number of novel noncoding RNAs.

Poster 25

ORF48 IS REQUIRED FOR OPTIMAL LYTIC REPLICATION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

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Kaposi's sarcoma-associated herpesvirus (KSHV) establishes persistent infection in the host by encoding a vast network of proteins that aid in the evasion of innate immunity pathways. The cGAS-STING is a potent immune sensor pathway associated with antiviral responses, and this pathway can inhibit the reactivation of KSHV from latency. Previously, we have identified multiple cGAS/STING inhibitors encoded by KSHV, which highlights the critical anti-KSHV role of this pathway and suggests the importance of these inhibitors on optimal KSHV lytic replications. In this study, we aim to further characterize one of these inhibitors, the KSHV ORF48. We hypothesize that ORF48 targets the cGAS/STING pathway to negatively regulate innate immunity, which will facilitate KSHV replication. We have constructed a set of recombinant viruses that included either wild-type (WT) or two ORF48-deletion viruses (KSHV.BAC16dORF48#1 or KSHV.BAC16dORF48#4). We then established iSLK cell lines that carry each of these viruses. We used doxycycline to reactivate KSHV into the lytic cycle in iSLK.BAC16 cells and harvested the cells at 0h, 24h, 48h, and 72h to assess the lytic replication status. We report that ORF48 deletion significantly abolished KSHV lytic replication in iSLK.BAC16 cells, as demonstrated by reduced levels of KSHV lytic gene transcripts, reduced KSHV lytic protein expression, reduced KSHV genome copies, and reduced number of infectious virions. Collectively, our data indicate that ORF48 is critical to optimal KSHV lytic replication. We are further dissecting the mechanism of ORF48 on innate immunity and KSHV lytic replication.

Poster 26

TITLE: Investigating the impact of bacterial extracellular vesicles on norovirus infection of macrophages

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ABSTRACT BODY: Human norovirus are the leading cause of gastrointestinal disease worldwide and are responsible for most diarrheal death in developing nations. Despite the global health burden caused by the pathogen relatively little is known about the mechanisms of norovirus pathogenesis. Recent studies have shown that commensal bacteria within the gut have the capacity to suppress and enhance viral replication in a region-specific manner. Commensal bacteria inhibit viral replication in the proximal small intestine, while simultaneously enhancing viral replication in the distal end, although the mechanism(s) for regulation of viral infection are still unclear. Our recent findings have shown that norovirus interacting with commensal bacteria lead to the increased production of bacterial extracellular vesicles (bEVs) in vitro and vivo providing the opportunity for these vesicles to influence viral infection. bEVs serve to suppress systemic and respiratory viral infection, but the role of bEVs is modulating infection of enteric viruses is unknown. To this end, we have now begun characterizing the impact of bEVs on various aspects of the norovirus infectious cycle. Specifically, are examining the influence of bEVs on murine norovirus (MNV) replication and host immune responses to MNV infection. Our results show that bEVs increase antiviral immune responses which coincides with reduced viral titers at the peak of MNV replication (18 hrs). We also found that bEVs, but not MNV, upregulate expression of *sting1* indicating bEVs may suppress MNV infection through the STING pathway. It has been previously shown that MNV does not induce or suppress STING during infection, therefore, bEVs may provide an alternate signaling pathway for induction of Type I IFN responses that ultimately lead to suppression of MNV replication. Understanding how bEVs induce the host to mediate unconventional antiviral pathways to suppress viral replication could provide a mechanistic framework for the identification of targets to prevent or treat norovirus infection.

Poster 27

Characterization of circ-virf4 in Kaposi's sarcoma-associated herpesvirus

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Circular RNA (circRNA) is a class of single-stranded RNAs that form a closed structure via backsplicing, which covalently joins the 5' and 3' ends. CircRNAs are relatively stable, resistant to exonucleases, and have been implicated in gene regulation and diseases, including cancer. Recent studies using RNase R-seq revealed the circRNAome of Epstein Barr virus and Kaposi's sarcoma-associated herpesvirus (KSHV), as well as interactions between KSHV and host circRNAs. The KSHV viral interferon regulatory factor 4 (vIRF4) region expresses mRNA and two circRNA isoforms with high expression in KSHV tumors, suggesting circ-vIRF4 may contribute to KSHV pathogenesis and/or tumorigenesis. To characterize the function of circ-vIRF4, a KSHV mutant lacking the splice donor site (Δcirc-vIRF4) was generated in the BAC16 bacmid. RT-PCR of Δcirc-vIRF4-infected iSLK cells shows that wild type isoforms are not detectable, but cDNA cloning from Δcirc-vIRF4 shows that alternative backsplice sites are used to express novel vIRF4 circRNAs. RNA-seq analyses comparing WT- and Δcirc-vIRF4-infected iSLK cells during latent or lytic replication demonstrated differential expression of both host and viral genes. Lytic libraries showed 1,494 differentially expressed genes (DEGs) and gene ontology (GO) analysis of host genes indicated roles in signal transduction, cell cycle, and cell migration. Analysis of latent libraries returned 569 DEGs, and GO analysis returned terms related to cell adhesion, cell differentiation, and development. These results suggest that a necessity exists for KSHV to express circRNA from the vIRF4 locus. Furthermore, circ-vIRF4 regulates host and viral gene expression during latent and lytic replication by an unknown mechanism.

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Tissue and cell tropisms of Zika virus in *Aedes aegypti* vector mosquitoes

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Zika virus (ZIKV) is a mosquito-borne flavivirus, which caused an explosive global epidemic in 2015. ZIKV is primarily transmitted to humans by the bite of an infected female *Aedes aegypti* mosquito. In mothers with confirmed ZIKV infection during the first trimester, 5 to 10 percent of infants were born with neurological birth defects. Since there are no vaccines or effective antivirals to control infections in humans, the mosquito stage of the transmission cycle is the most tractable for intervention. Despite this, central features of the viral life cycle in mosquitoes, like the receptors required for ZIKV entry, the cell types invaded by the virus in the midgut, ovary, and head cavity, and the antiviral effectors utilized by the mosquito immune system to destroy viruses remain unknown. Filling in gaps of understanding in the tissues and cell types targeted by these viruses during mosquito infection as well as the induction of immune mediated cell death or virus induced pathology is necessary to understand how these arboviruses evade mosquito immunity, and therefore augment their transmissibility and epidemic potential. This study aimed to improve understanding of the ZIKV life cycle in *Ae. aegypti* by determining tissue and cell type tropisms throughout the infection process, from the ingestion of an infectious blood meal to the mosquito becoming infectious by bite. We determined the prevalence of infection over time in ovary, midgut, brain, and salivary glands using whole mount immunofluorescence, to describe viral tropism and tissue barriers to infection. We used centrin and TUNEL counterstain, as well as transgenic mosquitoes expressing a fluorescent reporter protein under a pan-neuronal driver, to determine which cell types were infected and observe virus-induced pathology. We observed persistent infection of stem cells in the midgut and a tissue barrier to infection of the brain and ovary.

Poster 29

In vivo evolution of _env_ in SHIV-AD8-infected rhesus macaques after AAV-eCD4-Ig therapy

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Word Count: 298

eCD4-Ig is a fusion protein consisting of the ectodomain of CD4, an IgG Fc portion, and a short tyrosine-sulfated peptide that resembles the tyrosine-sulfated regions of all primate lentivirus coreceptors. eCD4-Ig mimics the engagement of both CD4 and CCR5 with the HIV Env, a property which imbues it with remarkable neutralization breadth. However, *env* is exceptionally genetically malleable and can evolve to escape a wide variety of entry inhibitors. Here we report the outcome of a pilot study that tested the ability of adeno-associated virus-mediated gene therapy with eCD4-Ig to suppress viral replication in viremic SHIV-AD8-infected rhesus macaques (RMs). Two of the five treated RMs developed persistent levels of eCD4-Ig in serum but experienced only partial or transient reductions in viremia. Longitudinal *env* sequencing performed over the course of 20 weeks revealed six mutations that became fixed at 100% frequency in plasma, two of which evolved independently in both animals. In one of the two RMs (47634), setpoint viremia plateaued at 1,000 vRNA copies/ml, despite concomitant serum concentrations of eCD4-Ig in the 70-100 ug/ml range. This unexpected phenotype prompted us to further characterize the *env* polymorphisms that evolved in 47634. We found that three mutations recovered from this animal (R315G, A436T, G471E) were sufficient to confer substantial resistance to eCD4-Ig-mediated neutralization on the parental SHIV-AD8 Env, accompanied by a marked cost to replicative fitness in host primary cells. These three mutations appear to confer eCD4-Ig resistance in part by taking advantage of a single amino acid difference between rhesus CD4 and eCD4-Ig (I39N) and in part by modulating CCR5 engagement, potentially allowing for use of alternate coreceptors. Taken together, our results shed light on the evolutionary pathways that remain open to *env* when it is confronted with a broadly neutralizing mimic of its major natural entry receptors.

Poster 30

Comparative transcriptome analysis of *Citrus macrophylla* tree infected with *Citrus tristeza virus* (CTV) stem-pitting mutants provide new insight into the role of phloem regeneration in stem-pitting disease

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Stem-pitting is a complex and economically important virus associated disease of perennial woody plants. The molecular mechanisms and pathways occurring during virus-plant interaction that result in this phenomenon are still obscure. Previous studies indicated that different *Citrus tristeza virus* (CTV) mutants induce defined stem-pitting phenotypes ranging from mild (CTVΔp13) to severe (CTVΔp33) in *Citrus macrophylla* trees. In this study, we conducted comparative transcriptome analyses of *C. macrophylla* trees infected with CTV mutants and full-length virus in comparison to healthy plants as control. Mild CTV stem-pitting mutant had very few differentially expressed genes (DEGs) related to plant defense mechanism and plant growth & development. In contrast, substantial gene expression changes were observed in plants infected with severe mutant and full-length virus. Analysis of transcriptome data for CTVΔp33 and full-length virus suggested that xylem specification has been blocked by detecting several genes encoding xylem, cell wall and lignin degradation, and cell wall loosening enzymes. Furthermore, stem pitting was accompanied by downregulation of transcription factors involved in regulation of xylem differentiation and downregulation of some genes involved in lignin biosynthesis, showing that the xylem differentiation and specification program has been shut off. Upregulation of genes encoding transcription factors associated with phloem and cambium development indicated the activation of this program in stem pitting disease. Furthermore, we detected induction of several DEGs encoding proteins associated with cell cycle re-entry such as chromatin remodeling factors, cyclin, and histone modification. This kind of expression pattern of genes related to xylem differentiation and specification, phloem and cambium development, and cell cycle re-entry is demonstrated during secondary vascular tissue (SVT) regeneration. Microscopy analysis confirmed that the regeneration of new phloem is associated with stem-pitting phenotypes. The findings of this study thus provide evidence that phloem regeneration contribute to the development of stem-pitting symptoms.

Poster 31

Unexpectedly high cross-reactivity between the receptor bind domains of SARS-CoV-2 and feline coronaviruses

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Abstract (299 words)

Feline coronavirus (FCoV) is one of the most common coronaviruses found in multi-cat households with prevalence ranging from 6.6% to 95%. FCoV differs from SARS-CoV-2 (SCoV2) in terms of target cells, pathogenesis, and clinical features, but both infect cats. Our team recently discovered that specific pathogen-free toms developed remarkably high levels of cross-reactive antibodies to the SCoV2 receptor binding domain (RBD) after mating with FCoV-positive queens, even though the amino acid (aa) similarity of their RBDs was low. Multi-aa sequence alignments of SCoV2 RBD, with four strains each from FCoV serotypes 1 and 2 (FCoV1, FCoV2), revealed 11.5% identity and 31.8% similarity with FCoV1 RBD, and 12.2% identity and 36.5% similarity with FCoV2 RBD. The sera from all three toms and three mated queens cross-reacted with SCoV2 RBD and also reacted with FCoV1 RBD but not with FCoV2 RBD. However, all sera reacted with spike-2, nucleocapsid, and membrane proteins of FCoV2 whole-virus. The plasma of all six FCoV2-inoculated laboratory cats reacted with FCoV2 and SCoV2 RBDs, but not with FCoV1 RBD. Furthermore, both serotypes of FCoV-infected cats, including FCoV1-infected group-housed laboratory and pet cats, produced the cross-reactive antibodies to SCoV2 RBD. Additionally, the SCoV2 RBD at a high non-toxic dose and FCoV2 RBD at a 60-400-fold lower dose prevented the *in vitro* FCoV2 infection of feline cells, demonstrating their close structural conformations essential as vaccine immunogens. In our preliminary human study, the sera from 14 COVID-19-positive unvaccinated patients had stronger cross-reactivity to FCoV2 spike-2 and nucleocapsid proteins than the 20 unexposed/unvaccinated humans, while the sera from 2/14 patients cross-reacted with FCoV2 RBD but none from the unexposed subjects. These results suggest that the cross-reactive epitope(s) on FCoV1 and FCoV2 RBDs may be similar to those of SCoV2 RBD and provide crucial insights into the development of a pan-CoV vaccine.