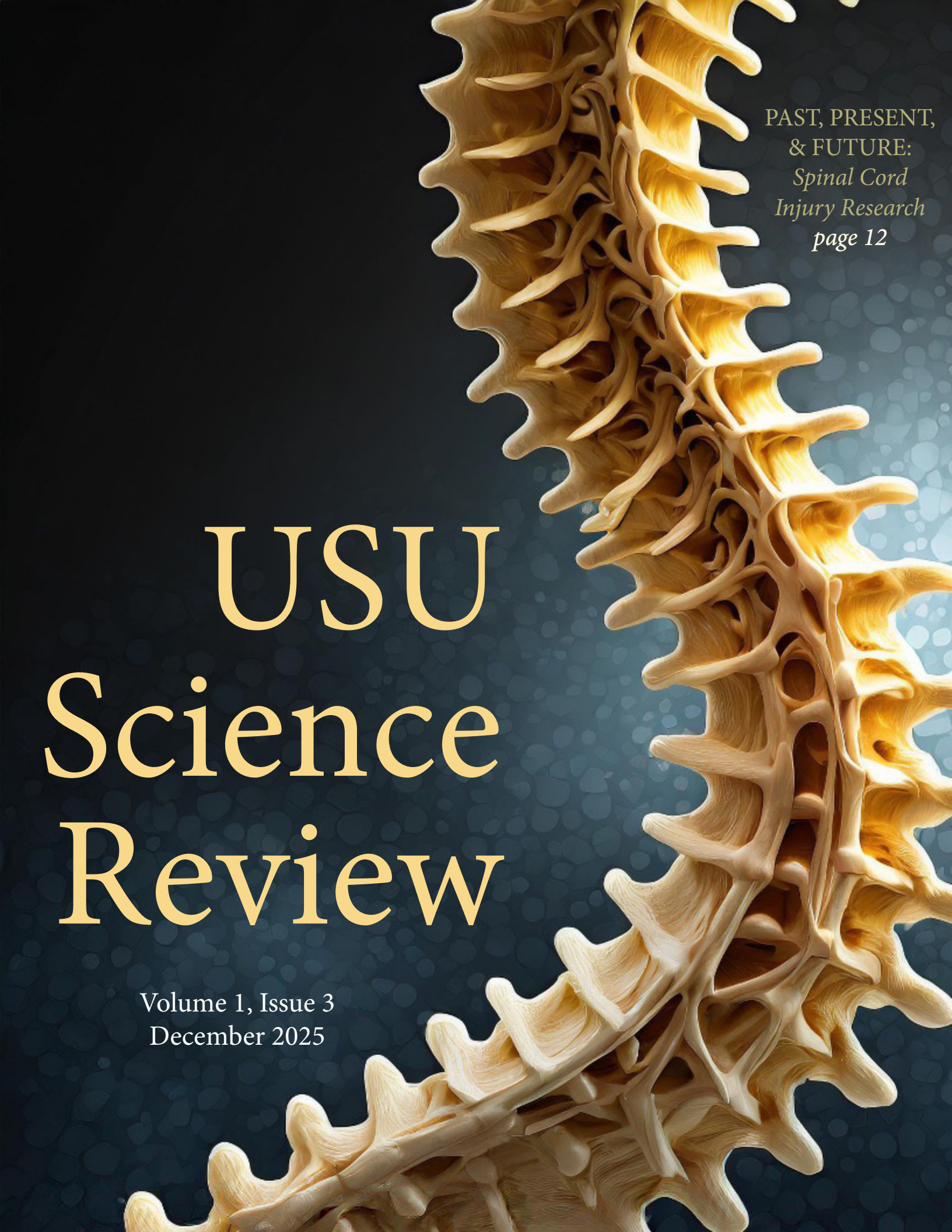

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FROM THE EDITORS

USU Science Review, an annual student-led joint faculty and student publication, has two principal functions: to foster intellectual discourse in the Uniformed Services University community, and to provide students with opportunities to develop their writing and editing skills. This third issue highlights the broad scientific work of the University, ranging from molecular biology to neuroscience, infectious disease, and global health as well as interests within professional development and health and wellness. Please note that articles from this issue were written in 2023 and reflect the current research at that time. We welcome any inquiries, feedback, and interest to get involved at srija.seenivasan@usuhs.edu, isabella.swafford.ctr@usuhs.edu, and alexandra.graninger.ctr@usuhs.edu. We hope you enjoy and learn from the talented voices in this issue.

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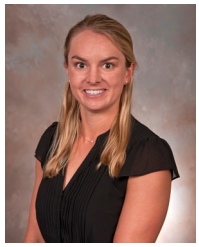

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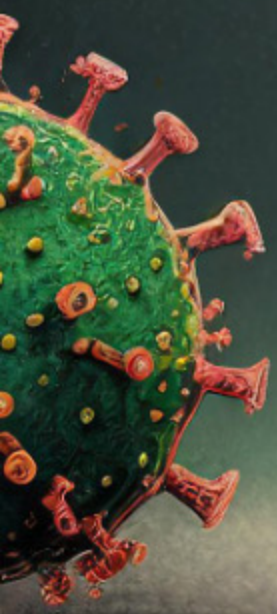
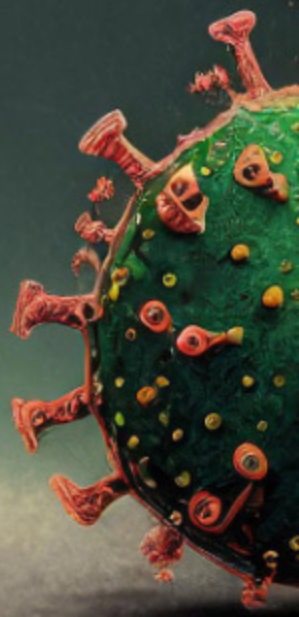
History of the Field

Thousands of scientific research papers are published every year, shedding light on the latest discoveries and novel modes of thought in our fields. With our hyperfocus on the latest products of science, we should not lose appreciation for the seminal discoveries and evolution of ideas that led us to our current scientific landscape. These stories from faculty experts share a historical perspective, including timelines of discoveries with technological, political, and economic challenges, providing appreciable context and reminding us that our individual contributions continue to drive this collective undertaking of discovery.

Vaccinology and the Battle Against Respiratory Viruses and the Next Pandemic

DR. ALLISON MALLOY





In 2023, a Nobel Prize was awarded for the development of a respiratory viral vaccine. Despite saving millions of lives each year, this was only the second awarded for a vaccine in the 122-year history of the Nobel Prize awards and, in the category of Physiology and Medicine, only the 13th awarded to a woman. While vaccines are often underappreciated, it is hard to ignore the impact vaccines have had on the COVID-19 pandemic. Less than one year after the discovery of SARS-CoV-2, a vaccine was available and an estimated 14.4 million lives were saved as of 2021 (1). COVID-19 vaccines, however, were a product of progressive developments and gains in the fields of virology, immunology, structural biology, and epitranscriptomics over hundreds of years. Therefore, while exposure to weakened pathogens has been a mainstay of vaccination, novel technologies and improved understanding of the immune response have dramatically changed the landscape for recent and future vaccines.

HISTORY OF INFLUENZA VIRUS VACCINATION

The development of respiratory viral vaccines required fundamental insights gained in the 1600s, and perhaps earlier, when exposure to smallpox through inoculation was implemented in the Caucasus Region and Ottoman Empire (2,3).

Despite causing smallpox in some recipients, skin inoculation, also known as variolation, was found to prevent disfiguring disease and death and became a widespread practice. Around 1796, Edward Jenner, and others, modified this concept by inoculating with cowpox, rather than smallpox, and this approach was found to be safer and relatively protective (4). This led to the term vaccination derived from the Latin word vacca, or cow. Using a modified or weakened virus has been the primary approach to immunization against influenza until more recently.

Influenza epidemics and pandemics were described as early as 412 BC from Greek texts, but it wasn't until 1933 that a group of British scientists first isolated an influenza virus after transmission to a ferret from human throat washings (5,6). The 1918 influenza pandemic was the first to be directly linked to an influenza virus; however, this was ultimately determined retrospectively with technology developed in the 21st century (7). The unusually high level of mortality in those 20-40 years of age associated with the 1918 flu resulted in reduced birth rates, available workforce, and military populations (8). The impact on society spurred research for a vaccination strategy and in the US, a scientific Commission on Influenza of the Armed Forces was established for this purpose in 1940. The commission was headed by Thomas Francis who, along with others in the field, demonstrated that live influenza virus administered subcutaneously in animal models did not result in disease and, when subsequently infected through the respiratory tract, provided protection (9).

Important in developing human vaccines was the discovery that viruses could be grown in eggs, facilitating growth and identification of influenza viruses (10). Influenza strains with high replication potential in eggs were selected for vaccines and formalin treatment became the primary method for viral inactivation. Initially these vaccines were not very effective but, by increasing the amount of inactivated virus administered, a reduction in transmission and disease severity was observed (11). The study of viral growth in chicken eggs also demonstrated that influenza virus caused agglutination, or clumping, of red blood cells (12). Binding of an influenza protein to erythrocytes was determined to be the cause of the hemagglutination; this protein was appropriately named hemagglutinin (HA). Identification of HA and the discovery that viral strain-specific antibodies could prevent hemagglutination enabled the development of an assay to identify new strains of influenza.

Enhancing exposure to HA to increase

These included split-virus vaccines, which used a detergent to disassociate the viral lipid envelope and expose all viral proteins, and subunit vaccines, which contained HA and neuraminidase (NA) proteins alone (13). However, it became clear that mutations in HA resulted in evasion of prior vaccine- or infection-induced antibodies. To limit viral evasion, the number of viral strains included in the vaccine was increased. For the 2023-2024 influenza season vaccine, two A strains and two B strains were included. The H1N1 A strain was updated from the prior year, while the H3N2 was unchanged. Neither of the two B strains were changed from the prior season and despite not circulating for the last two seasons, the B\Yamagata strain was maintained.

Inaccurate predictions of circulating influenza strains and viral mutations have resulted in failures of the influenza vaccine program and are the focus of current research. Reliance on eggs for vaccine production requires 6-9 months of preparation and contributes to the inaccuracy of strain selection. In addition, changes in the virus can occur during the growth process in eggs, altering antibody binding sites. Historically, egg allergies were a contraindication to the flu vaccine further reducing the number of people who could be vaccinated. However, research showed that the filtration process mitigated this contraindication and in 2023-2024 this policy was changed.

The challenges with manufacturing and viral mutations have led to multiple approaches to improve the design and manufacturing of influenza vaccines. Mammalian cell lines have been used for the growth of influenza strains, reducing production-induced mutations, shortening replication time, and relieving the pressure of ensuring sufficient numbers of eggs. In 2007 and 2012, vaccine strains grown in cell lines were approved in Europe and the US, respectively (14). Advancements in sequencing provided an opportunity to avoid replication of viral strains. Instead, the HA sequence was inserted into a baculovirus expression system, resulting in a recombinant HA protein vaccine that was approved in 2013 (15). Further advancements in subunit, nanoparticle, and mRNA vaccines have provided additional platforms to improve manufacturing and potentially increase antibody levels. Mucosal vaccination with live-attenuated vaccines potentially increases respiratory mucosal antibody and cellular responses at the sight of infection and has been used since 2008. None of the current platforms, however, circumvent the seasonal strain variation that requires annual updates to the vaccine. Identification of antibody targets on the more conserved portion of the HA protein are actively being sought to create universal flu vaccines that would provide protection across strains (16).

HISTORY OF RESPIRATORY SYNCYTIAL VIRUS VACCINES

Respiratory syncytial virus (RSV) was first identified in chimpanzees suffering from respiratory illness at the Walter Reed Army Institute of Research in 1955 (17). Subsequently, RSV was detected in young infants with lower respiratory disease and recognized to be a ubiquitous pathogen of childhood (18,19). Once isolated and the burden of disease ascertained, plans for vaccine development were approached similarly to influenza and polio. RSV, however, required recovery in primary human cells or cell lines. A throat swab obtained from a patient at the National Institutes of Health and inoculated into human embryonic kidney cells yielded the isolate for the first RSV vaccine. Subsequently, the virus was passaged in monkey kidney cell cultures, filtered, formalin-inactivated, and precipitated with alum. By the 1960s, the Division of Biological Standards had established testing for complement fixation and antibody production, which was performed in guinea pigs and cynomolgus

monkeys. Evaluation for local and systemic adverse reactions were then performed in adult humans. The vaccine product, named Lot 100, was then tested in children under two years of age from low socioeconomic backgrounds and from families stationed at Fort Ord, California (20,21). Unexpectedly, young infants who had been immunized with the formalin-inactivated (FI) RSV had an 80% hospitalization rate compared to control vaccinees who had an 8% rate of hospitalization. Additionally, two recipients of the formalin-inactivated vaccine died.

This tragedy prompted research into the cause of FI-RSV-associated severe disease, which has led to advances in the fields of immunology, virology, and genetics. In order to understand this adverse outcome, new animal models were established and our understanding of CD4 T cell differentiation and use of T helper type 2 immune responses by infants advanced. The role of antiviral antibodies was also intensely studied, leading to the discovery that immunoglobulin enriched for RSV antibodies could reduce severe disease (22,23). These studies were performed in the 1990s at USUHS, led by Dr. Hemming, and resulted in the discovery of monoclonal antibodies that could neutralize RSV. Palivizumab was approved in 1998 as a monthly injection for preterm infants during the RSV season.

Further advancements in RSV vaccines required an improved understanding of protein structure. As crystallography advanced, the structure of the F and G surface glycoproteins could be mapped for antibody binding sites (24). Vaccines using subunits, chimeric viruses, vector-delivery systems, virus-like particles, and nanoparticles for antigen presentation began being developed. Antibodies binding a prefusion structure of the F protein were found to have a higher neutralization potential and became the focus of new vaccine design (25). The prefusion F structure was unstable, however, and required new techniques for stabilization in order to be provided as an immunogen. Techniques established to stabilize prefusion F led to the development of the recently licensed subunit vaccines that are now approved for adults over the age of 60 years and pregnant women (26,27). Identification of highly neutralizing antibodies generated against the stabilized prefusion F protein also led to the development of the monoclonal antibody, Nirsevimab, that was modified to have a 3-6 month half-life and is now approved for administration to all infants during their first RSV season (28). While no vaccine currently exists for infants and young children, leaving gaps in protection, the current products will hopefully reduce the burden of disease in the youngest and most vulnerable infants, as well as those over 60 years of age.

HISTORY OF SARS-COV-2 VACCINES

In 2019, a novel coronavirus was identified in China that soon spread across the globe. While therapeutics were sought, a path to vaccination began immediately. In December of 2019, the sequence of the Spike protein, the viral entry and fusion protein of this new coronavirus, was provided to experts in vaccine design around the world. Knowledge of protein structure and stabilization developed for RSV was then applied to the virus that became known as SARS-CoV-2. Established animal models were used to rapidly identify neutralizing antibodies and determine if vaccination with a stabilized prefusion Spike protein could protect against infection. However, vaccinologists knew that production of a stabilized prefusion Spike protein for mass vaccination strategies would take too long. Techniques to use the human body's machinery to make proteins were then considered for more rapid vaccine development. Since 2002, Dr. Katalin Kariko had been working on modifying messenger RNA (mRNA) to produce proteins in dendritic cells for antigen presentation to T cells to fight cancer (29). Dr. Kariko's work was undertaken in

in collaboration with Dr. Drew Weissman at the University of Pennsylvania. Together they discovered that modification of nucleosides with a pseudouridine could reduce excess inflammation and increase translation of synthetic mRNAs (30). This breakthrough led to the development of the COVID-19 vaccine and their receipt of the Nobel Prize for Medicine and Physiology in 2023. While the COVID-19 vaccine was available less than a year after SARS-CoV-2 was discovered, the research that resulted in its production was decades in the making.

FUTURE OF RESPIRATORY VIRAL VACCINES

Centuries of progress resulted in the development of a vaccine that could be rapidly deployed in a pandemic. The research behind modern vaccines, in some ways, also impairs the full potential of vaccination. The establishment of influenza growth in eggs allowed for mass production of vaccines, but has also resulted in a manufacturing challenge that we have yet to overcome. The limited protection of human study participants through the 1980s continues to impair the public's opinion of vaccines and prevent vaccine uptake. While we have made tremendous advancements in vaccinology, we also have many unanswered and new questions regarding mucosal immune responses, correlates of protection, and the goals of vaccines. In the next century, pandemics will likely be met with the knowledge we are currently seeking.

DR. ALLISON MALLOY IS A PEDIATRIC INFECTIOUS DISEASES PHYSICIAN-SCIENTIST AND AN ASSOCIATE PROFESSOR IN THE DEPARTMENT OF PEDIATRICS. DURING HER RESIDENCY AT THE CHILDREN'S HOSPITAL OF PHILADELPHIA, SHE BECAME INCREASINGLY CURIOUS ABOUT THE EVOLUTION OF THE HUMAN BODY WITH AGE. INFECTIOUS DISEASES PROVIDED A UNIQUE LENS ON HOW PATHOGENS COULD TEACH US ABOUT OUR IMMUNE SYSTEM AND THE TRAJECTORY OF HEALTH. SHE JOINED DR. BARNEY GRAHAM'S LAB AT THE NIAID VACCINE RESEARCH CENTER TO STUDY THE IMMUNE RESPONSE TO RESPIRATORY SYNCYTIAL VIRUS (RSV) AND ESTABLISHED HER RESEARCH FOCUS ON RESPIRATORY MUCOSAL IMMUNITY TO VIRUSES. DR. MALLOY'S RESEARCH PROGRAM AT USU CURRENTLY STUDIES SEVERAL RESPIRATORY PATHOGENS INCLUDING RSV, INFLUENZA, AND SARS-COV-2.

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Spinal Cord Injury
Research and Treatment
PAST, PRESENT, AND FUTURE

Dr. Kimberly Byrnes

Devastating consequences of spinal cord injury

As of 2023, there are approximately 302,000 people living in the United States with a spinal cord injury (SCI), and about 18,000 new injuries occur every year (1). An SCI is defined as any damage to the spinal cord, the bundle of axons and supporting glia, as well as the central gray matter of neurons inside the spinal cord, which transmits information between the central and peripheral nervous system. SCI disrupts this signaling, causing loss of the brain's ability to communicate with the body and the body to communicate with the brain.

Demographically, most SCI's happen in men (70%) and in two age brackets: young (16 – 25) and aged (70 – 80) (2). As the population has aged and older people are more active, the average age for SCI has steadily increased from approximately 30 in the 1990s to 43 currently. Depending on the age bracket, most SCIs are the result of sports (young) or falls (aged). The area of damage dictates the type and extent of injury, with 50% of cases occurring in the cervical region, and 50% of cases occurring in the thoracic/lumbar.

There are several consequences to the spinal cord tissue after injury. Depending on the type of damage, whether the spinal cord was crushed by bone or other object, transected by a bullet or other weapon, or contused briefly by bone, the injury sequelae varies in timing and severity of response. In general, the injured spinal cord demonstrates a marked loss of blood flow and sudden onset of ischemia, as well as loss of connectivity of descending or ascending axon tracts, increased inflammation, and local neuronal death (3). If left unchecked, the lesion can slowly expand over the course of the individual's life, contributing to permanent loss of motor and sensory function.

Initial therapeutics and clinical trials

Scientists have been seeking a cure for SCI for decades, if not centuries, with the earliest published studies into therapeutics starting in the late 1960's. Prior to that, injury to the spinal cord was often considered hopeless and unable to be treated (4). Faced with SCI's incurred during World War I, the neurosurgeon Harvey Cushing indicated that almost 80% of those with injury would not survive more than a few weeks. Those with acute SCI, including President James Garfield and General George Paxton passed away from complications of SCI within weeks of injury. However, neurosurgeons and other doctors began to show promise in SCI treatment in the early 20th century, with spinal cord traction and treatment of secondary complications after injury (4). Rehabilitation centers began to grow in popularity and improved outcomes by the 50's in Europe, Australia, and eventually the US.

The early research studies often used naturally occurring SCI in dogs and focused on surgical decompression, hypothermia, and glucocorticoids, which were found to improve motor function (5). Thirty years later, when I entered the field, a considerable amount of research had built upon that work, and new therapeutics had been introduced, but, somewhat surprisingly, surgical decompression and glucocorticoids were still the top targets for therapeutic approaches after SCI.

Surgical decompression, removal of the vertebra or debris above the spinal cord contusion or compression injury, has since become the most common approach for SCI treatment (6).

In the late 1990's and early 2000's, methylprednisolone (MPSS), a corticosteroid known for having strong anti-inflammatory and antioxidant effects, was well on its way to becoming the gold standard for SCI therapy. The National Acute Spinal Cord Injury Studies (NASCIS) performed in the late 1980's and early 1990's demonstrated that MPSS had prominent neuroprotective effects in rodents after SCI (3). In 2010, however, MPSS started to fall out of favor. Between 2000 and 2010, an increasing number of studies began to show that MPSS administration was tied to high rates of pneumonia and sepsis (3). By 2010, only about 20% of patients received steroids like MPSS (7). In 2013, the American Association of Neurological Surgeons (AANS) recommended against MPSS for SCI, due to the potential for significant harmful side effects.

In the 1990's to early 2010's, a number of additional therapeutic approaches appeared in the literature, targeting a number of aspects of the injury response, such as the glial scar, axonal regeneration failure, and inflammation. Treatments to reduce inflammation or the glial scar and associated proteins that block regeneration improved outcomes in a number of animal models (8-14). However, few made it to clinical trials, and none were successful in human trials. To confront this challenge, the North American Clinical Trials Network (NACTN) was established in 2004 by Dr. Robert Grossman in collaboration with SCI survivor and activist, Christopher Reeve, with funding from the Christopher and Dana Reeve Foundation and the Department of Defense (3) to facilitate clinical translation, standardize clinical care, and improve SCI outcomes.

Present day SCI therapies and research

The NACTN is still in operation today, consisting of 9 centers including Walter Reed National Military Medical Center (WRNMMC). Over the years the NACTN has been responsible for creating a database of clinical trial data, incorporating other associations and independent trials worldwide to facilitate data sharing. Its database now stands with data from over 1,000 patients, including their responses to various clinical trials.

Where is the bench now with research? There is a drive now to develop a deeper understanding of mechanism and epidemiology of SCI, in order to better tailor treatments to patients and improve clinical trial success. Transcriptomics, genomics, and deeper investigations into cells play a key role in current SCI research.

At the bedside, patients are benefiting from years of improvement in decompression surgery (15). The survivability after SCI has dramatically improved over the last 5 decades, with mortality within the first 2 years after injury dropping by 40% since the 1960's (16). The AANS has issued a number of new recommendations that are designed to improve both recovery and life expectancy, including hypotension treatment, strict vaccination schedules, aggressive physiotherapy for both internal organs and motor functions, and diet and bowel management programs. In early 2020, the NIH held a workshop to declare that the 2020's would see advances toward a cure for SCI. In addition, they invited SCI survivors to share what they wanted from scientists, and the early 2020's, as a result, have seen an increase in research and treatments for the non-central effects of SCI, such as autonomic dysreflexia, bowel and bladder dysfunction, sexual dysfunction, peripheral immune compromise, and cardiac and pulmonary problems, which survivors have indicated as their priorities.

In addition to these peripheral effect studies, therapies targeting the spinal cord itself are on-going. Therapies focusing on neuroprotection (minocycyline, riluzole, hypothermia, specifically to the spinal cord, immunomodulators), neuroplasticity (epidural or vagus nerve stimulation), regeneration (cethrin, biomaterial scaffolds, NOGO), and replacement of function (cell transplants, robotic exoskeletons) are in early clinical trials or late translational trials (15). Currently, SCITrials.org and the clinical trials government website list over 400 actively recruiting interventional trials for SCI, including interventions such as epidural stimulation, stem cell transplantation, glyburide, hyperbaric oxygen, pulsed magnetic stimulation, exoskeleton, aggressive physical therapy, hypothermia, and more.

Work by scientists like Gregoire Courtine, Phil Popovich, Monica Perez (the 2024 National Neurotrauma Society [NNS] keynote speaker), and more have shown that recovery after SCI is possible. Dr. Courtine's most recent work has shown that brain-machine interfaces in humans with SCI can lead to walking, in a paper that, 20 years ago, would have seemed more science fic-tion than science (17). And clinical trials, such as the Riluzole trial started in 2013, RISCIS, have begun to show promise. While still underpowered, the initial results show that all cervical SCI patients treated with riluzole, a sodium-glutamate antagonist, significantly improved functional recovery after treatment (3).

Speaking to SCI survivors at study sections and survivor meetings, or in advocacy sessions at meetings like the NNS symposium, reveals that many have experienced marked improvements in their function following stem cell transplants and utilization of epidural stimulators. Many patients are reporting a considerable amount of hope and excitement for the direction that SCI research and treatment is heading.

The future of SCI treatment

Many scientists and clinicians see a world of potential for SCI. Advances in understanding the molecular underpinnings of the spinal cord's response to injury and treatment have opened

a vast field for therapeutic development. Research into sex and age influences are beginning to shape recruitment for clinical trials, to allow for better understanding of therapeutic utility at the individual rather than population level.

Focus on the peripheral effects of SCI and treat-ment approaches for non-spinal cord targets will steadily improve the quality of life for SCI survivors. And as SCI survivors live to later ages, more research into the chronic SCI man-agement and treatment will further improve life expectancy and outcomes.

SCI researchers are banding together, through programs like the NACTN and others, as they understand that collaborative science and data sharing are keys to furthering SCI treatment and care. Open Data Commons-SCI, the data sharing platform for SCI, is now online (odc-sci.org), and sharing data from over 122 labs and 235 datasets. This open sharing approach to science will, hopefully, lead to faster and more coordinated progress into restoring function and quality of life for SCI survivors.

SCI is a complex, difficult to treat condition involving a number of different cell types and the ability of the brain to communicate with the body. Scientists are exploring a number of avenues to restore this communication, and the future may see a number of these come to full fruition. A future of brain-powered exoskeletons that allow SCI survivors to live independently, supplemented with direct spinal cord stimulation that allows a survivor to bypass the damaged part of a spinal cord. Advanced medications that promote regeneration or autologous stem cell transplants that allow for replacement of last cells could also be on the horizon, leading to the elimination of SCI as a chronic dysfunction of the brain and body.

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DISCOVERY OF THE ENDOPLASMIC RETICULUM

Dr. Jessica Rosarda

The cellular compartment now known as the endoplasmic reticulum (ER) was initially discovered more than a century ago. In 1890, Gustav Retzius and Santiago Ramon y Cajal independently observed a reticular structure in cells using a silver chromate reaction. This approach was combined with light microscopy by Emilio Veratti in 1902 to illuminate the fine details of a reticulated organelle adjacent to the Golgi apparatus (1). Studies on the ER languished until it was revisited in 1945, when Keith Porter, Albert Claude, and Ernest Fulla combined osmium tetroxide staining with transmission electron microscopy (TEM) to show high-resolution images of a 'lace-like reticulum' in the endoplasm from which 'vesicle-like bodies emerged' (1,2). George Palade famously went on to elucidate the role of this "endoplasmic reticulum" within the secretory pathway using novel radioactive labeling and cell fractionation techniques (3,5). Our understanding of ER structures continues to build upon the seminal works of Palade and others published from the 1950s through the 1970s.

Their work revealed that nearly one-third of the cellular proteome localizes to the ER for fold-ing, processing, sorting, and secretion (6). The ER consists of a single membrane that surrounds a lumen, forming interconnected sheets and tubules that extend from the nuclear membrane to the cell periphery (7, 8). Sheets are flat with curved regions located only at the edges. The rough ER (RER) is primarily composed of sheets; it is described as "rough" because small ribo-nucleo-protein particles, called ribosomes, are embedded in the cytosolic membrane layer (8, 9). Ribosomes synthesize polypeptides from amino acids to form proteins. Newly synthesized polypeptide chains are co-translationally transported from the ribosomes into the ER lumen, where they proceed through protein folding and quality control (QC) processes (5). Downstream of the RER, the 'smooth ER' or ER-Golgi intermediate compartment (ERGIC), is primarily composed of highly curved tubules (8, 10). From the ERGIC, proteins are packaged into vesicles via the Golgi and then sorted to their respective membrane compartments or secreted (7, 10).

Endoplasmic reticulum functions within the cell

From the early 1970s onwards, scientists began connecting ER structures to their cellular functions using mutagenic screens. By examining the phenotypes of genetic mutants from screens in the budding yeast Saccharomyces cerevisiae, researchers linked numerous cellular proteins to their respective ER processes (11). The RER sheets, with their larger lumenal spaces, were found to mostly contain protein folding factors, such as chaperones (10). The curved tubules of the smooth ER were more involved in metabolism, such as lipid production, and specialized tasks including calcium storage and detoxification (7). Parallel efforts in mammalian systems revealed that this sub-compartmentalization of ER functions was highly conserved across eukaryotic lineages (5, 11).

Studies in metazoans suggested that the presence of rough or smooth ER in a cell correlated with the level of secretory activity of that particular cell type (7). The amount of rough and smooth ER within a cell is determined by the same transcriptional programs that drive cell specialization (7, 12). Following differentiation, distinct cell types exhibit varying basal capacities for ER protein folding and metabolic functions as required for their biological functions (12). For example, healthy pancreatic beta cells have a large RER that allows for the secretion of up to one million molecules of insulin per minute (13). In contrast, Leydig cells produce large quantities of steroid hormones and possess larger smooth ER structures (14). Overall, specialized cell types tend to require specific ER structures to fulfill their proper function.

While the basal capacity for ER functions is determined by the cell type, cells can also expand the size and capabilities of their ER in response to environmental stimuli (12, 15, 16). When the metabolic or protein folding demands on the ER exceed its capacity, otherwise known as ER stress, a defined set of signaling pathways known as the Unfolded Protein Response (UPR) are activated (15, 16). Activation of the UPR helps to manage ER stress by functioning as a feedback loop. UPR activation decreases the protein folding load while increasing the size and functional capacity of the ER. UPR activity then diminishes as ER homeostasis is restored. Because the demand for ER functions is constantly shifting in response to intracellular or extracellular cues, the UPR is critical for maintaining cellular physiology in response to homeostatic perturbations (16, 17).

The Mammalian Unfolded Protein Response

The UPR in mammals is a more complex and diversified system compared to the pathway first discovered in yeast by Jeffery Cox and Peter Walter in 1996. Cox and Walter showed that the UPR of budding yeast is a straightforward signaling cascade controlled by the ER stress activated protein IRE1 (18). In contrast, the mammalian UPR contains a homolog to the yeast IRE1, as well as the transcriptional and translational responses downstream of the ER stress sensing proteins PERK and ATF6 (19). Together, IRE1, PERK, and ATF6 regulate global protein synthesis and the expression of thousands of genes involved in ER functions (19). All three pathways can be activated by common sources of ER stress, such as calcium flux or increases in the abundance of unfolded or misfolded proteins (16, 20). However, more recent studies also revealed specific cellular factors or chemicals that selectively activate individual UPR pathways, demonstrating the independent functioning of the three ER stress sensing proteins in regulating distinct cellular processes (21, 22).

A variety of approaches for modulating these pathways have helped to define the arm-specific impacts of PERK, IRE1, and ATF6 activity on ER function (20, 23, 24). PERK activation inhib-

its global protein synthesis, which decreases the number of nascent polypeptides to be folded in the ER and also promotes the expression of genes for amino acid synthesis (23, 25). The activation of IRE1 increases the expression of ER quality control machinery and degradation factors to reduce the abundance of misfolded proteins in the ER and can promote inflammatory signaling in some models (20, 23, 26, 27). ATF6 activity enhances the expression of protein folding chaperones, redox factors, and sterol synthesis enzymes that function to fold ER proteins properly and expand the ER membrane network (20, 23, 28).

Whether activated collectively or individually, signaling from PERK, IRE1, and ATF6 function to remodel the ER environment, restore protein homeostasis, and regulate cellular metabolism during stress conditions (16, 20, 23). When UPR activity is insufficient to overcome ER stress, cellular functions become severely impaired; thus, under-activation of the UPR can be damaging to cells and tissues. However, severe or persistent UPR activation can also cause cell death and tissue damage by promoting pro-inflammatory or pro-apoptotic signaling downstream of IRE1 and PERK activity, respectively (24, 25). As a result, both the under- and over-activation of UPR signaling has been implicated in the pathology of numerous human disorders, including developmental, metabolic, and neurodegenerative diseases (17, 24, 25, 29).

Contributions of the ER to human disease

Links between ER function and human diseases have been recognized for nearly 75 years. As early as 1956, clinicians observed hypertrophic expansion of smooth ER structures in the liver in response to a variety of toxins and injuries (30-32). However, in 1968 investigators found that, hypertrophic ER structures eventually became hypoactive, leading to organ failure. More recent studies have found that this adaptive exhaustion occurs in other disorders associated with ER stress (22, 33, 34). In these models, persistent or repeated stresses ultimately exhaust the pro-adaptive functions of the UPR, leading to cellular apoptosis and reductions in tissue integrity (22, 31, 33, 34). However, the precise mechanism by which this occurs remains poorly characterized for most disorders.

ER stress and its role in pathology are perhaps best understood in ER protein misfolding disorders. In these diseases, the stability of proteins folded in the ER is compromised due to genetic mutations or ER stress triggered by injury, disease, or toxins (35, 36). When these destabilized proteins are targeted for degradation, the loss of protein function disrupts normal cellular processes, leading to disease (36, 37). Alternatively, insufficient ER quality control can permit destabilized proteins to form cytotoxic protein aggregates (35-37). While activation of the UPR can remodel the cellular environment to prevent protein aggregation by limiting synthesis and increasing degradation, these processes can also exacerbate loss of function toxicity (35). More recent efforts have focused on the selectively amplifying or muting the effects of individual arms of the UPR to mitigate the pathologic ER stress (24).

Pharmacologic modulators of UPR pathways potential as therapeutic interventions

The development of chemical screening approaches and compound libraries has accelerated the discovery of pharmacologic modulators of ER protein homeostasis, or proteostasis. The first compound found to mitigate ER stress induced cell death, Salubrinal, was discovered in 2005 by screening ~19,000 compounds. Salubrinal prevents ER-stress induced apoptosis by prolonging the activation of PERK-regulated signaling path-ways and has been shown to be protective in disorders where insufficient PERK signaling contributes to pathology (24, 25, 38). More

recently, selective activators of IRE1 and ATF6 signaling were discovered (26, 39). Activators of IRE1 reduce the cytotoxic aggregation of amyloid precursor protein (APP) and improve glucose-stimulated insulin release from the pancreatic beta cells of obese mice (24, 26, 40). Pharmacologic activators of ATF6, discovered in 2016, have now been shown to alleviate pathology in various disease models, including the visual disorders achromatopsia and Macular Telangiectasia II; cardiac, kidney, and cerebral I/R injury; epilepsy, and spinal muscular atrophy (SMA) (22, 24, 28, 41).

For disorders where hyperactive UPR signaling contributes to disease pathogenesis, a number of pharmacologic inhibitors of the UPR pathways have been discovered. Pharmacologic inhibitors of signaling downstream of PERK, such as ISRIB, have been shown to improve memory functions and reduce pathology in models of the neurologic disorder Vanishing White Matter (VWM) disease (25, 42, 43). Inhibitors of IRE1 signaling, such as 4u8C and STF-083010, have been shown to reduce pro-inflammatory signaling downstream of IRE1 activity in models of atherosclerosis and dyslipidemia (24, 27). Pharmacologic inhibitors of ATF6 have not demonstrated protection in any models of disease; instead, these compounds tend to increase the sensitivity of cells to ER-stress induced toxicity (22, 28). Of the UPR inhibiting compounds, pharmacologic inhibitors of PERK signaling seem the most likely to be approved for use in clinical settings.

Future directions for the field

Our understanding of the role of the ER in cellular physiology and disease pathology will continue to improve as molecular, biochemical, and imaging technologies advance. At USUHS, my laboratory will be working to understand why some cell types, such as hippo-campal neurons and retinal photoreceptors, are uniquely sensitive to some types of cellular stress, including ER stress (22, 44-46). My previous work suggests that the sensitivity of cells to ER stress may be dictated by cell type-specific factors associated with ER function (22, 46, 47). By using techniques such as single nucleus sequencing, we aim to identify specific cell types that are uniquely sensitive to stress in different disease contexts. In addition, we will use molecular screening methods to discover new pathological sources of ER stress in stress-sensitive cells. Further, we will apply genetic, chemical-genetic, and pharmacologic approaches for modulating UPR and other signaling pathways to determine the extent to which these compounds promote protection or worsen damage. Through these efforts, we seek to identify promising new therapeutic approaches for mitigating cellular and ER stress in a variety of diseases relevant to members of the military and veteran communities.

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Student Scientific Reviews

Literature reviews are crucial resources for scientists. This section provides an opportunity for USU students to provide critical analysis of published literature and present a current comprehensive summary of their field of interest.

Mind Your Ts and Qs

Allison Ruchinskas

T cells are an intrinsically dynamic arm of the adaptive immune response, given their role in responding to foreign antigens and subsequently shaping the immune response through their effector functions. As a vital component of the immune system, T cells become activated upon engagement of their T cell receptor (TCR) with a cognate antigen (presented as peptide:MHC), along with costimulation through CD28 (1, 2). Activated CD8+ cytotoxic T cells can recognize and kill infected cells presenting the antigen. CD4+ T helper (Th) cells function to recognize and respond to antigen by differentiating into discrete subsets that assist other immune functions. Each Th subset arises from a unique transcriptional program that commits the recently activated T cell to produce specific cytokines that deputize other immune cells and tailor the immune response. Th fate is influenced by TCR signal strength and the local cytokine profile (3). Importantly, the growing field of immunometabolism argues that metabolite availability may be just as influential in defining the lineage of these Th cells (4, 5).

Once a CD4+ T cell recognizes its cognate antigen with appropriate costimulatory signals, the newly activated T cell rapidly switches its metabolic signature (5). The predominant shift is from energy production dependent on oxidative phosphorylation to aerobic glycolysis. Although less efficient for ATP production, glycolytic intermediates provide macromolecules necessary for T cells to proliferate and acquire effector functions. Therefore, disruptions in metabolite availability can alter the trajectory of T cell differentiation.

T cell activation is accompanied by TCR-induced upregulation of multiple nutrient transporters for glucose, amino acids, and iron. Of the amino acid transporters, SNAT1, SNAT2, ASCT2, and LAT1 are all significantly increased upon TCR ligation. Whereas SNAT family transporters drive initial clonal expansion, ASCT2 and LAT1 appear more vital for differentiation (6, 7). The latter two transporters work together: ASCT2 imports glutamine (Q) from the extracellular environment, and the LAT1 antiporter uses some of that increased intracellular glutamine to transport leucine into the cells (7, 8). In addition to enabling LAT1 function, imported glutamine feeds directly into several pathways that synthesize macromolecules like nucleotides, amino acids, fatty acids, and glycoproteins (9, 10). Of note, glutamine is an essential metabolite in generating an inflammatory Th1 response, which is crucial for cell-mediated immunity against viruses and intracellular bacteria (5, 11). Naïve Th cells lacking glutamine cannot differentiate into Th1 cells but can still become Th2 cells, which classically combat worm and parasite infections (11). Yet misdirected Th2 responses to innocuous substances drive allergy, emphasizing a potential link between metabolite restriction and preferential, pathogenic Th differentiation (12). Delineating how metabolites and their transporters regulate Th differentiation is not only essential to understanding these dynamic processes but could provide an avenue for novel therapies. Notably, glutamine supplementation can modulate immune responses to prevent or shift detrimental allergic states (13-16). However, we currently have a limited understanding about which TCR-dependent signaling pathways are necessary for increased glutamine uptake and how glutamine can contribute to this shift.

Our lab examines how cell signaling and metabolism influence human lymphocyte function, often in the context of inborn errors of immunity (IEI). Interrogating primary cells from IEI patients coupled with in vitro genetic and pharmacological manipulations to model their disease will provide valuable insights into normal and dysregulated T cell biology. In particular, we study immune dysregulation in patients harboring mutations in CARD11, a scaffold protein that links TCR stimulation with several essential downstream signaling pathways: nuclear factor kappa B (NF-kB),

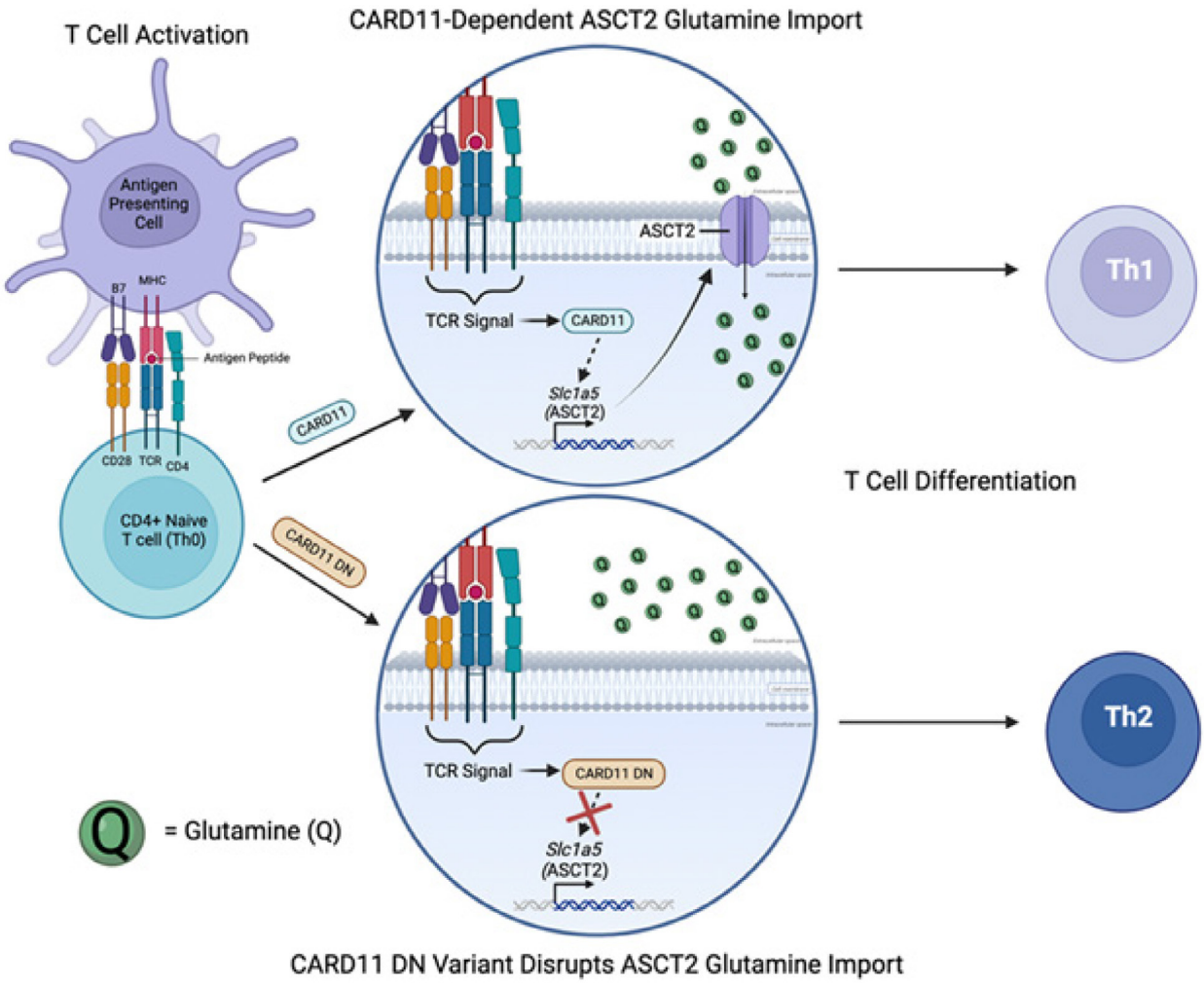

Figure 1. Working model for the role of CARD11 in ASCT2 expression and function. CARD11 DN mutations could interfere with glutamine import thereby shifting newly differentiating CD4+ T cells to a Th2 phenotype.

c-Jun terminal kinase (JNK), and mammalian target of rapamycin complex 1 (mTORC1) (1, 2, 12, 17-20). NF-kB is a crucial transcription factor for T cell proliferation and survival, whereas JNK and mTORC1 are implicated in T cell differentiation, with mTORC1 governing metabolism and protein translation. Specifically, we concentrate on patients with heterozygous loss of function (LOF) dominant negative (DN) CARD11 mutations that impair the ability of wild-type CARD11 to form a functional signalosome and potentiate TCR and costimulatory signal (12, 21, 22). Strikingly, 90% of these patients exhibit severe allergic symptoms, which arise from their Th cells either defaulting to or preferentially skewing toward an aberrant Th2 phenotype (12, 17, 21, 22). Could abnormal glutamine import hold the key to understanding this pathology?

Previous studies in mice demonstrated that ASCT2 induction upon T cell activation requires CARD11 expression (7). Our studies focus on dissecting the contributions of CARD11 and its signalosome partners in regulating ASCT2 expression and function in human T cells. Interestingly, CARD11 DN patient T cells show decreased levels of ASCT2 overall and delayed upregulation of ASCT2 upon activation (21). We hypothesize that attenuated ASCT2 expression and subsequent disruption of glutamine import contributes to the Th2 accumulation observed in CARD11 DN patients (Figure 1). Determining the downstream CARD11-dependent pathway that controls TCR-dependent ASCT2 expression can help us discover therapeutic targets and delineate specific pathway impacts in CARD11 DN patients.

The link between glutamine availability and T helper differentiation is also poorly understood. We posit that glutamine availability, which is essential for mTORC1 function, is dependent on CARD11 function downstream of TCR signaling (23-25). mTORC1 is a multifunctional nutrient-sensing complex; the respective function of mTORC1 and its sister complex mTORC2 influences the adoption of particular Th subsets, with inflammatory Th types (like Th1) requiring mTORC1 function (3, 26-28). We know CARD11 contributes to TCR-induced mTORC1 activation, though the molecular mechanism remains unclear (18). If our hypothesis is correct, we can link TCR-induced, CARD11-dependent mTORC1 function to ASCT2-mediated glutamine uptake.

Current diagnostic assays within our group can swiftly determine if novel CARD11 variants affect NFKB and JNK signaling after TCR stimulation (21, 22). Increasing evidence highlighting the importance of nutrient import and mTORC1 function in T cell fate mandates further exploration of how CARD11 variants can also impact this pathway. Furthermore, examining the contributions of TCR signal strength, CARD11, glutamine, and mTORC1 to Th differentiation is foundational to understanding diseases driven by an aberrant Th response. Glutamine supplementation may provide a simple, effective intervention to restore a Th1 response in patients like those carrying CARD11 DN variants. Ultimately, targeting metabolism and nutrient availability could present an attractive therapeutic option for patients with abnormal Th cell differentiation and ameliorate symptoms in a growing number of individuals suffering from severe allergies.

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Exploring the HPA axis:
A Hunt for PTSD-associated sex-dependent neurochemical alterations

Mydirah Littlepage-Saunders

Among the US population, 50%-80% of people will experience at least one traumatic event in their lifetime, yet, only roughly 7% will develop post-traumatic stress disorder (PTSD)[1]. The heterogeneity of PTSD, comorbidities, and wide array of overlapping symptoms with other medical conditions make it a complicated disorder to study. Hence, there is a lack of a full understanding of the pathophysiology of the disorder and limited treatment options. Epidemiological studies suggest environmental and biological variables make certain populations more vulnerable to developing PTSD. Sex is one identified risk factor, given that women are more than twice as likely to develop PTSD than men[2].

When examining sex differences, analysis of gonadal hormones, especially estrogen, is common among preliminary research. Estrogen has been implied to have a leading hormonal influence on the female brain and behavior, and clinical data has associated low estrogen levels with anxiety disorder vulnerability[3-6]. While multiple studies have examined the role of estrogen in PTSD, other hormones and neurosteroids associated with the hypothalamic-pituitary-adrenal (HPA) axis are also being investigated. The HPA axis, made up of the hypothalamus, pituitary gland, and adrenal cortex, is the key driver of the stress response and adaptation. In response to stress, the hypothalamus releases corticotropin releasing hormone (CRH) which activates the anterior pituitary and induces the release of adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal cortex to synthesize and secrete cortisol (or corticosterone in rodents). This neuroendocrine system, which connects perceived stress to physiological reactions, has been associated with PTSD both clinically and preclinically[7].

Analyzing differences in blood plasma and cerebrospinal fluid (CSF) levels of known HPA axis markers between PTSD patients and healthy individuals has aided in identifying primary drivers of PTSD vulnerability. Functional neuroimaging (i.e fMRI) offers advanced capabilities to clinically characterize neural markers and circuits driving PTSD. However, preclinical models are valuable and necessary to investigate and manipulate region-specific neurochemicals that cannot be assessed clinically. Rodents are commonly used in preclinical PTSD research, where a plethora of paradigms exist: restraint, inescapable shock, predator-stress, social defeat, social isolation, and single prolonged stress. These PTSD models rely on stress-induced onset of PTSD-like symptoms (i.e increased freezing, impaired fear extinction, avoidance). Here we will review different neurochemicals that impact the HPA-axis and could influence the sex difference seen in the prevalence of PTSD and differences in symptomology.

CRH: CRH is the central regulator and initiator of the cascade of events that occur along the HPA axis. Clinical studies have identified elevated levels of CRH in the CSF of veterans with PTSD compared to traumatized veterans without PTSD and healthy controls[8, 9]. However, that study was conducted in males only and no similar studies have examined CRH levels in women to determine whether a sex difference in CRH is present. It has been demonstrated clinically that CRH is higher in healthy young women than men, regardless of circulating estrogen levels[10]. An increase in CRH concentration has also been seen in the CSF and brain regions associated with processing emotions (i.e. amygdala) and storing memories contextualizing emotions (i.e hippocampus) in both stress- and glucocorticoid-exposed subjects; this has been demonstrated in preclinical and clinical studies[11]. One preclinical study examined the correlation between resilience to social stress and DNA methylation of the CRH gene in male mice. This study found that following social defeat, the subset group of mice that displayed resilience did not have increased CRH mRNA levels in the PVN, which has projections from the amygdala, unlike

the subset group of mice that demonstrated avoidance following social defeat[12]. Furthermore, this study showed that social avoidance, a proxy for a symptom of PTSD, was able to be prevented by decreasing expression of CRH using small interfering RNA (RNAi)[12].

There are sex-specific differences in expression and function of CRH[13]. The timing of stress exposure, whether it is pre-, during, or post-development, can also play a role in the sex-dependent effects of CRH. For example, one study found that trauma-induced avoidance is pronounced in adult female mice, regardless of whether CRH is overexpressed in early life. Yet, adult male mice only displayed pronounced avoidance if they had been exposed to early-life overexpression of CRH[14]. The same study found that following stress and/or CRH overexpression, there were sex-specific alterations to the genes Fkbp51 and Crhr2, which play a role in regulating CRH and glucocorticoids[14].

Glucocorticoids: Glucocorticoids, the end product of HPA axis activation, are released from the adrenal cortex as the classic endocrine response to stress. The endogenous glucocorticoids are cortisol, in humans, and corticosterone, in rodents, which bind to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). One study investigated sex differences in cortisol levels at baseline and in response to stress, by giving ACTH injections, and found that both free cortisol and ACTH-stimulated cortisol release was higher in males[15].

Glucocorticoid receptors are expressed throughout the brain but at higher levels in the hippocampus, amygdala, and prefrontal cortex[16]. Mineralocorticoid receptors are predominantly expressed highest in the hippocampus[10]. These regions have circuitry level ties to the neuroendocrine system and the facilitation of behavioral responses to stress[10, 16]. Genetic analysis has shown the polymorphisms for GR- and MR-related genes, NR3C1 and NR3C2, have sex-dependent susceptibility to neuropsychiatric symptoms[17]. Polymorphisms to the GR-related gene, NR3C1, are threefold higher in women[18], while specific haplotypes to MR-related gene NR3C2 are associated with depression in women but resilience in men[19].

Rodent models have revealed sex-differences in glucocorticoid secretion. Contrary to clinical studies, resting corticosterone levels were reported as higher in female rats than male rats[20]. Another study showed that female rats have higher stress-induced plasma corticosterone levels than their male counterparts[21]. Multiple studies have found that the expression of GRs and MRs is lower in female rats, compared to males, in both the pituitary and hypothalamus[22-24].

Dehydroepiandrosterone (DHEA): DHEA is an endogenous steroid hormone that is also produced in the adrenal cortex and co-released with glucocorticoids. Previous research suggests that DHEA counters the actions of cortisol, exerting anti-oxidant and anti-inflammatory effects and acts as a resilience factor against severity of PTSD symptoms[16, 25]. Clinically, a low DHEA to cortisol ratio projected more severe PTSD symptoms, while a higher ratio correlated with symptom improvement[26]. Being that DHEA is a precursor to sex hormones, a sex difference in expression is expected. Studies have consistently reported significantly higher CSF DHEA levels in men compared to women[27]. Since DHEA may buffer the severity of PTSD, this would support the higher prevalence of PTSD in women[28].

The neuroprotective effects of DHEA on PTSD vulnerability have been demonstrated in preclinical studies, where administration of DHEA resulted in antidepressant-like effects in rodent models[29-31]. In terms of DHEA expression, preclinical studies consistently have not found a sex difference but identify a main effect of age, where DHEA declines in both sexes over time[32]. There are limited preclinical rodent studies that examine DHEA, considering it is much less abundant in rodents compared to humans and primates. Hence, a different preclinical model may be needed to determine the mechanism of action of DHEA and whether it has a sex-dependent effect on PTSD vulnerability.

Allopregnanolone: Clinical reports show an inverse correlation between the levels of DHEA and allopregnanolone, a neurosteroid and allosteric modulator of GABA-A receptors known to inhibit the HPA axis [33]. Downregulation of allopregnanolone is associated with increased PTSD re-experiencing symptoms and comorbid depressive symptoms in humans[34]. A postmortem study revealed only males diagnosed with PTSD showed a significant decrease in allopregnanolone compared to healthy male counterparts, the females did not show a significant difference when comparing diagnoses[35].

Preclinically, low allopregnanolone levels correlates to enhanced contextual fear memory and impaired fear extinction[36, 37]. A study that examined the impact of acute swim stress on neuroactive steroid concentrations in rats showed sex-dependent changes for allopregnanolone, where females displayed higher basal plasma and brain concentrations and that swim stress increased allopregnanolone concentrations more in females than the males[38]. This contradicts the negative correlation between allopregnanolone and PTSD phenotypes. However, the study measured allopregnanolone at the peak time for stress-induced allopregnanolone. Allopregnanolone negatively modulates the HPA axis[39]. Therefore, other timepoints should be assessed to observe whether there is a sex difference in recovery time, as it is possible that females may have increased levels for a longer duration than males which could suggest feedback dysfunction.

Selective Serotonin Reuptake Inhibitors (SSRIs): Serotonin (5HT), commonly recognized as the "happiness hormone", plays a key role in mood regulation. Low levels of serotonin have been associated with mood disorder symptoms such as depression and anxiety. A clinical study showed in vivo serotonin synthesis rates are higher in men compared to women[40]. SSRIs are the only FDA-approved pharmaco-treatment for PTSD, however many individuals are non-responsive to SSRIs and are characterized as having treatment-refractory PTSD. Clinical studies suggest that women are more responsive to SSRIs[41-43]. Considering low 5HT is associated with PTSD and women have shown slower 5HT synthesis, SSRIs would decrease the need for 5HT synthesis supporting why SSRIs are more beneficial for PTSD treatment in women.

Preclinical studies suggest a sex-dependent serotonergic contribution to HPA axis activation that is greater in females compared to males. One study showed that administration of the SSRI citalopram increased corticosterone significantly faster and had a longer recovery rate in female mice compared to males[44]. Another preclinical study found that serotonin depletion, by knocking out its precursor tryptophan, enhances fear response[45]. Other than SSRIs ability to make serotonin readily available at synapses, preclinical studies have demonstrated that these pharmacological agents also increase allopregnanolone levels, resulting in normalized fear response and fear extinction[33, 46, 47].

Neuropeptide Y (NPY): While the research on serotonergic systems weakly presents resiliency towards PTSD and PTSD-like phenotypes, NPY unveils a protective role against stress. NPY is abundant throughout the limbic system, especially the hypothalamus, and has multiple functions, including fear and stress[48]. A clinical study that assessed combat-related PTSD saw significantly reduced NPY concentrations in the CSF of male veterans diagnosed with chronic PTSD compared to healthy men[49, 50]. Unfortunately, the majority of clinical PTSD studies have primarily been conducted in men, and those that have used both men and women have not analyzed NPY levels. Despite the lack of baseline comparison of NPY across sexes, one clinical study showed that NPY increases with age which aligns with the clinical data suggesting the younger the age of trauma exposure increases the risk of PTSD development in females[51-53].

The results of preclinical studies align well with the clinical research demonstrating NPY as a resiliency factor against PTSD-like symptoms. One study found a significant increase in freezing in both NPY knockout (KO) and NPY Y1 KO mice, but not NPY Y2 KO mice, emphasizing that NPY facilitation, specifically mediated through NPY receptor Y1, enhances fear expression and impairs fear extinction[54]. Other studies showed that administration of NPY and Y1 agonists resulted in antidepressant-like effects in rodents following different single prolonged stress protocols[55, 56]. However, the aforementioned studies only used male rodents. Few, but some studies looked at sex differences in NPY expression. One study suggests sex-specific expression of NPY, where female rodents displayed lower blood NYP levels and had less NPY-expressing neurons[48]. The hypothalamus was the primary region where male rodents had increased NPY levels than females which could be due to testosterone's ability to stimulate the release of NPY in the arcuate nucleus that is located in the mediobasal hypothalamus[57, 58].

Although several neurochemical and behavioral deficits have been recapitulated between preclinical and clinical studies, an optimal translation to human neuropathology or neuroimaging is still missing. While environmental factors that increase PTSD prevalence in populations such as the military may be hard to mimic preclinically, the ideal preclinical model should encompass sex as a biological variable to showcase the significant role it has on the etiology of PTSD.

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Dual nature of *Enterococcus faecalis*: Commensal and pathogen

Prati Gurung

Enterococci, Gram-positive bacteria, are ubiquitous microorganisms found in water, plants, soil, foods, and the gastrointestinal tracts of humans and animals (1). These facultative anaerobes exhibit a remarkable ability to thrive in various environmental conditions, including extremes in pH, temperature, and salt concentrations (2). This exceptional tolerance plays a crucial role in the ability of Enterococci to colonize diverse host environments, persist in different settings, and serve as indicators of fecal contamination (3). Renowned for their lactic acid production, they have historically been utilized in food fermentation and biopreservation due to their enzymatic activities, proteolytic traits, and the production of antimicrobial peptides (4,5). Additionally, enterococci have been explored as probiotics, offering various benefits such as immune stimulation, anti-inflammatory effects, hypocholesterolemic influence, and the prevention or treatment of certain diseases (6). However, recent years have witnessed a significant debate surrounding their use in food and as probiotics. This debate stems from concerns of opportunistic pathogenicity linked to virulence factors and antibiotic resistance, notably with the emergence of vancomycin-resistant strains (7). Enterococci display inherent resistance to numerous antimicrobial agents and have the ability to acquire resistance determinants encoded on a diverse range of conjugative plasmids, transposons, and bacteriophages (8,9). The virulence traits exhibited by *Enterococcus faecalis* and other species are associated with genetic transfer mechanisms, further complicating their role as commensals. Among these enterococci, *E. faecalis* has become a significant player in healthcare-associated infections (HAIs) in recent decades, contributing to endocarditis, central line-associated bloodstream infections, catheter-associated urinary tract infections, and surgical site infections (1). *E. faecalis* functions both as a beneficial commensal and as an opportunistic pathogen, a duality worth exploring.

E. faecalis characteristics and role as a commensal

Enterococci, initially discovered in human fecal flora in 1899, were historically considered part of the *Streptococcus* genus. *Streptococcus faecalis* was first documented in 1906 when it was isolated from a patient with endocarditis (10). Subsequently in 1984, a division occurred within serogroup D streptococci, prompted by differences observed in biochemical characteristics and nucleic acid properties. This division resulted in the renaming of the isolate as *E. faecalis,* making it part of newly created *Enterococcus* genus, which now encompasses over 50 different species (10,11).

Human microbiome analysis indicates that there are around 5000 microbial species, ranging across 24 phyla and 2000 genera. Among them, up to 65% of gut bacteria are Firmicutes, which include enterococci. It's estimated that between 10^6 and 10^7 Enterococcus bacteria, primarily *E. faecalis* (at 10^5 – 10^7 CFU/g feces), reside in the human intestine, with less than 1% found in the ileum and up to 1% in the colon (12). As a commensal, *E. faecalis* plays a role in metabolic processes, pH regulation, and the synthesis of vital nutrients. The gut microbiota is seen as a virtual endocrine organ, where *E. faecalis* plays an immunomodulatory role and is responsible for the activation of CD4, CD8 (CD-cluster of differentiation) cells, and B lymphocytes (12,13). *E. faecalis*, along with other commensal bacterial species, plays a crucial role in the early colonization of the infant gut, acquired from the mother's flora (14). Like other enterococci, *E. faecalis* is able to produce bacteriocins known as enterocins, which are small peptides with antimicrobial properties. These enterocins are active against closely related Gram-positive bacteria. including notable pathogens such as *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes,* and *Staphylococcus aureus* (15,16). In summary, *E. faecalis* as a commensal aids in nutrient metabolism, participates in immune regulation,

prevents pathogen colonization, and contributes to a diverse and healthy gut microbiota.

Probiotics are live microorganisms with beneficial health effects when consumed adequately, primarily consisting of lactic acid bacteria (LAB) like Bifidobacteria and Lactobacilli, with occasional use of enterococcal species, particularly *E. faecalis* (6). These probiotics have different applications in the pharmaceutical industry, human and veterinary medicines, and the food industry. Numerous studies confirm the probiotic attributes of *E. faecalis,* showcasing significant health-promoting effects. *E. faecalis* strains, such as those found in FortiFlora®, Cernivet®, and Symbioflor® 1, serve as effective and safe food supplements (17-19). These enterococcal probiotics demonstrate potential in treating or preventing human and animal diseases, including relieving irritable bowel syndrome symptoms, mitigating antibiotic-induced diarrhea, and preventing various intestinal conditions. Notably, *E. faecalis* strains also exhibit additional favorable effects, such as antimutagenic, anticarcinogenic, hypocholesterolemic, and immune regulation properties (20,21).

Nonetheless, *E. faecalis* can migrate from the gut to other parts, form biofilms, readily acquire various virulence factors, and develop resistance to a range of antibiotic classes. With numerous reports on its genetic adaptability and potential for pathogenic traits, the question arises: how imminent is the transformation of *E. faecalis* from a commensal species to a pathogenic one?

Virulence factors and biofilm formation

Previous studies have shown that *E. faecalis* inhibits macrophage apoptosis and enhances their survival, irrespective of bacterial strain. It also suppresses the immune response, potentially facilitating infection with other pathogens. Additionally, in high doses, *E. faecalis* delays wound healing and reduces inflammatory cytokine expression in mice (22-25). *E. faecalis* exhibits virulence through key determinants, including aggregation substances, cytolysin, extracellular surface proteins, adhesion to collagen, endocarditis antigens, and gelatinase (4). Aggregation substances aid in bacterial conjugation and colonization. Cytolysin acts as a peptidic toxin and is associated with higher mortality rates in infections. Extracellular surface proteins facilitate adhesion and immune evasion. Genes encoding collagen adhesion enhance virulence and the virulence gene *efaA* plays a vital role in endocarditis. Gelatinase contributes to hydrolysis, biofilm formation, and tissue damage (6). In addition, *E. faecalis* readily forms biofilms, which are structured microbial communities consisting of bacterial cells embedded in a self-produced extracellular matrix of polysaccharides, proteins, and DNA that provides protection and support (26,27). Biofilms are formed when individual bacteria adhere to surfaces, multiply, and secrete this matrix, which provides protection and support. Enterococci contain multiple biofilm-associated virulence factors, including the transcriptional regulator AhrC and the metalloprotease Eep (28). *E. faecalis* biofilms contribute to a higher percentage of catheter-related urinary tract infections, are frequently identified in wound infections, and are becoming more prevalent in cases of infective endocarditis (1,26,29). These pervasive communities are particularly concerning because biofilm populations are estimated to be 10 - 1000 times more resistant to antibiotics than planktonic cells. This enhanced resistance results from multiple mechanisms, such as the presence of persister cells and reduced antibiotic penetrance.

Antibiotic resistance

The rising development of antibiotic resistance in enterococci, particularly hospital *E. faecalis* isolates, is a growing concern. *E. faecalis*, known for its inherent resistance and ability to acquire and transfer resistance genes through horizontal gene transfer, poses a notable threat (30,31). For instance, the VanA enterococci, including *E. faecalis*, demonstrate high-level resistance to vancomycin, a critical first-line antibiotic. Vancomycin- resistant enterococci (VRE) is a growing concern when it comes to *E. faecalis* infections, with hospital expenses ranging from $33,224 to $124,257 per patient (32). Furthermore, there is evidence suggesting their role in transferring vancomycin resistance genes to *Staphylococcus aureus* and other nosocomial pathogens (33). *E. faecalis* adeptly navigates the host's defense mechanisms, notably evading the immune system through intrinsic resistance to lysozyme. Lysozyme, a potent antimicrobial enzyme found in bodily secretions and immune cells, usually effectively targets bacteria (34,35). However, *E. faecalis* employs multiple resistance mechanisms. For example, the metalloprotease Eep is instrumental in the SigV-mediated lysozyme resistance pathway. By cleaving RsiV, an anti-sigma factor, Eep facilitates SigV release after lysozyme

sensing at the cell surface (Figure 1). These intricate mechanisms underscore the robust resistance of *E. faecalis* against lysozyme, encompassing a network of factors and pathways (34,36-38).

Ongoing research

Excessive antibiotic use has spurred the evolution of multidrug-resistant bacteria like *E. faecalis*, highlighting the potential of antimicrobial peptides such as lysozyme for combating pathogens. Understanding lysozyme resistance mechanisms is crucial for effective strategies. Thus, my research project revolves around investigating eep-related lysozyme resistance in *E. faecalis*. In addition to providing lysozyme resistance, Eep is crucial for several other functions in *E. faecalis*, such as biofilm formation, peptide signaling, and the transfer of antibiotic resistance. To better understand the resistance mechanisms of *E. faecalis*, we have been studying a laboratory strain called OG1RF, which has no mobile genetic elements or plasmids (39). This makes any genetic determinants discovered in this strain more likely to be shared by other *E. faecalis* strains. By deleting *eep* (Δ*eep*), we identified lysozyme-sensitive (LysS) strains, but also isolated lysozyme-resistant (LysR) strains, all lacking *eep*. Whole genome sequencing of a LysR Δ*eep* strain revealed a lysozyme susceptibility suppressor mutation in *liaX*, a gene in the LiaFSR system linked to adhesion and antimicrobial (daptomycin) sensing. My goal is to characterize the contribution of *liaX* in *eep*-related lysozyme resistance.

Currently, my study delves into the unique resistance patterns and growth dynamics of the *E. faecalis* double mutant Δ*eep*Δ*liaX*, which exhibits partial resistance to lysozyme alongside increased sensitivity to detergent and bile salt. Both Δ*eep*Δ*liaX* and Δ*liaX* mutants display heightened resistance to antibacterial compounds like polymyxin B and nisin compared to OG1RF, with delayed growth dynamics observed in single and double mutants. Additionally, aggregation occurs earlier in Δ*liaX* and Δ*eep*Δ*liaX*. These findings highlight the intricate interplay between Eep and LiaX, suggesting potential synergistic effects. Understanding lysozyme resistance mechanisms could offer valuable insights for potential treatment strategies against *E. faecalis* infections. One such strategy involves combining lysozyme with other antibiotics and antimicrobials, which could enhance killing of *E. faecalis*, thereby complementing antimicrobial efficacy.

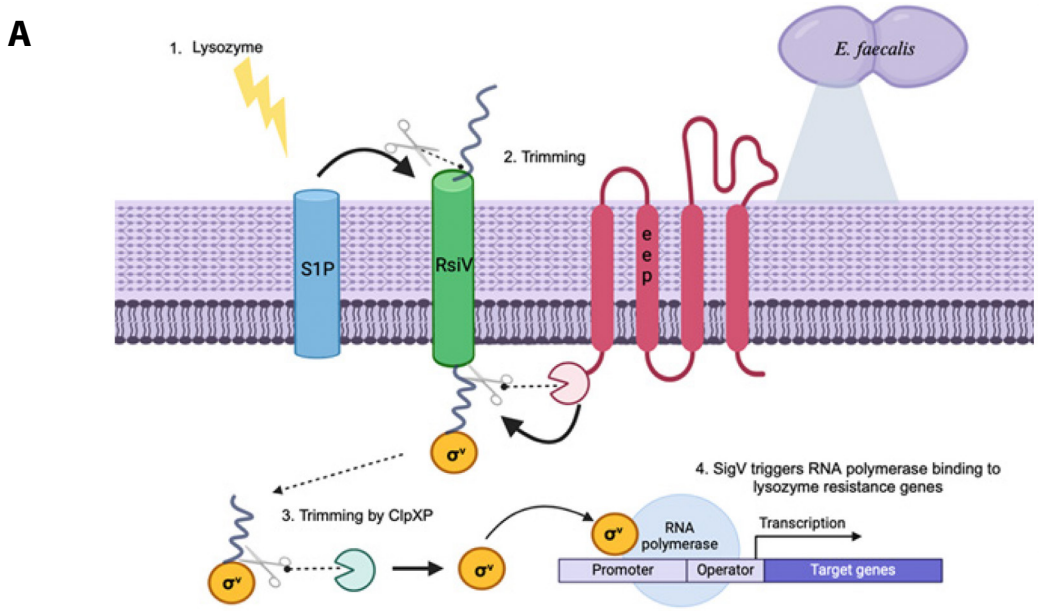

Figure 2. A role for Eep in the regulation of lysozyme resistance genes. A model for regulated intramembrane proteolysis (RIP) of RsiV involves a series of proteolytic steps culminating in the activation of SigV (σv), its associated sigma factor. First, when *E. faecalis* encounters lysozyme stress, RsiV undergoes cleavage by a putative site 1 protease (S1P). Additionally, *E. faecalis* possesses additional trimming protease activity that modifies RsiV for targeting by Eep. Subsequently, Eep degrades the processed and trimmed RsiV, releasing SigV into the cytoplasm. Then, ClpXP, a cytoplasmic protease, further breaks down RsiV, facilitating the release of active SigV. Finally, SigV facilitates the binding of RNA polymerase to specific genes that enhance lysozyme resistance.

The dual nature of *E. faecalis*, serving as both a commensal organism and a potential pathogen, poses a crucial dilemma. It prompts us to consider the challenge of selectively targeting the pathogenic aspects of *E. faecalis* while preserving its beneficial commensal attributes for gut health. It also raises the question of whether the risks associated with it being a commensal organism are too significant to overlook. Current research efforts are focused on discerning how quickly *E. faecalis* can transition into a pathogenic one without clear warning. Notably, my project places a specific emphasis on understanding the role of lysozyme resistance in this dynamic process. In essence, current research seeks a nuanced understanding of the relationship between *E. faecalis*, the gut environment, and the impact of antimicrobial resistance on its commensal and pathogenic behaviors, offering insights for potential therapeutic interventions.

Pratibha Gurung is a 5th year PhD candidate in the Emerging Infectious Diseases Program. She is in Dr. Kristi L. Frank's laboratory in the Department of Microbiology and Immunology at USU where she researches mechanisms by which Enterococcus faecalis, a Gram-positive bacterium that is both a human commensal and an opportunistic pathogen, develops resistance to antimicrobials like lysozyme.

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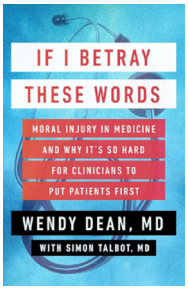
Book Club

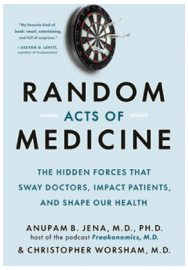
Content for scientists, curated by scientists.
The USU community shares reviews of books and podcasts they enjoyed for the next like-minded individual on the hunt for a good read/listen.

FOR THE READERS



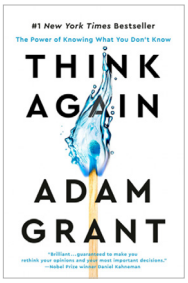
If I Betray These Words
by Wendy Dean
Contributor: Dr. Monica Lutgendorf

This was a great book discussing some of the challenges facing physicians in medicine and a thorough and diverse evaluation of issues surrounding moral injury in healthcare, where clinicians are unable to provide the care their patients need, and the effects of rising costs of care on patients. I found some really thought provoking themes that I think are important for us to consider.



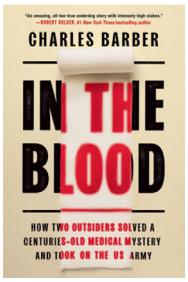
Random Acts of Medicine
by Anupam B. Jena and Christopher Worsham
Contributor: Dr. Emily Ricker

Does timing, circumstance, or luck impact your health care? This groundbreaking book reveals the hidden side of medicine and how unexpected—but predictable—events can profoundly affect our health. • Is there ever a good time to have a heart attack? Why do kids born in the summer get diagnosed more often with A.D.H.D.? How are marathons harmful for your health, even when you're not running?



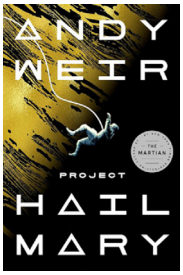
Think Again by Adam Grant
Contributor: Anonymous

As important as it is to think and learn, it is at least as important to unlearn and rethink. It is challenging to open our minds to rethinking when we are so confident in our knowledge, but only by rethinking are we truly maintaining a growth mindset and committing to life long learning.



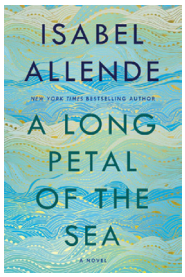
In the Blood: How Two Outsiders Solved a Centuries-Old Medical Mystery and Took On the US Army
by Charles Barber
Contributor: Dr. Krista Highland

After 9/11, the Marine Corps and Navy turned to QuikClot, an inexpensive product to control hemorrhage. The Army went with "Factor Seven," a product with deadly consequences. Ultimately, Dr. Ian Black, an Army anesthesiologist, became a whistle-blower, which resulted in a large investigation and lawsuit by the Department of Justice.



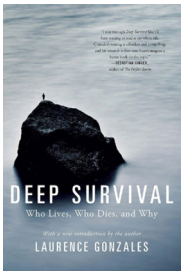
Project Hail Mary by Andy Weir
Contributor: Lauren Haacke

An astronaut wakes up with amnesia from an induced coma on a spacecraft and must put together why he is there, what he is meant to do, and why the two people next to him are dead. His memories weave the past with the present as he realizes just how existential this mission is.



A Long Petal of the Sea by
Isabel Allende
Contributor: Aisha Lomax

A Doctor finds himself in the middle of a civil war in Spain. A wonderful reflection of medical professionals and displaced migrants due to war.



Deep Survival: Who Lives, Who Dies, and Why by Laurence Gonzales
Contributor: LTC Patrice Shanahan

Discusses physical and psychological (emotional and cognitive) characteristics that lead people to survive or die in austere environments, accidents, and dangerous situations. He followed people who have dangerous jobs and adventurous hobbies reporting on the mechanisms that help them survive or lead to their demise. A must read for military!

FOR THE LISTENERS

All There Is by Anderson Cooper
Contributor: Yessenia Gomez

Exploration of loss and grief

Shakespeare vs Milton: the Kings of English Literature Debate by Intelligence Squared
Contributor: Karen Williams

A fascinating debate on the merits of two renegade writers in western literature.

Conferences

In the past six months, USU students and faculty attended scientific meetings on a variety of topics in locations all over the world. Here, they recount their personal experiences and newfound knowledge, and share reviews of the conferences they attended.

Please note that articles from this issue were written in 2023 and reflect the conferences at that time.

1 AMERICAN COLLEGE OF OBSTETRICIANS & GYNECOLOGISTS (ACOG) ARMED FORCES DISTRICT (AFD) ANNUAL MEETING

Claire Sturek, SOM Medical Student
September 24-27, 2023 Tacoma, WA

I am so grateful that I had the opportunity to attend the American College of Obstetricians and Gynecologists (ACOG) Armed Forces District (AFD) Annual Meeting in September of 2023. ACOG was founded in 1951 and continues to serve obstetricians and gynecologists at the national and local levels. The organization publishes practice guidelines for physicians, videos for medical students and residents, and educational material for patients. It also facilitates programs to improve women's health, provides career support, and advocates for members and patients. It is composed of 12 districts representing various regions, countries, territories, and states in North and South America. The Armed Forces District (AFD) is most relevant to students at USUHS, as it is composed of Army, Navy, and Air Force physicians and represents unique research interests and clinical needs within the military community.

I attended as a third-year medical student to present research about the development of a mobile app. My team developed the Pre-Exposure Prophylaxis (PrEP) Resource, an app that guides physicians through initiating, routinely following, and discontinuing PrEP. The Centers for Disease Control and Prevention (CDC) estimates that only 10% of eligible women received appropriate PrEP therapy in 2019, so this study was relevant to the target patient population. With the encouragement of my mentors, I applied for the Gibbons Award which provided funding to attend the event in Tacoma, Washington. Their guidance also helped shape the poster and manuscript we submitted, which was recognized with the Outstanding Poster Award.

The event was a great platform to share my team's work, learn about the ongoing research in the field, and meet Gynecologic Surgery and Obstetrics (GS&O) physicians practicing in all three services. There was a healthy mix of work and play, with the highlight being the "Seattle Grunge" themed Jeopardy, in which the three services were tested on their medical knowledge. I am proud to share that the Navy won! Presenting at ACOG AFD showed me the nurturing environment within the ACOG community and its commitment to the development of future leaders in women's healthcare. I would highly recommend for third and fourth-year medical students interested in GS&O to attend this conference.

AMERICAN PSYCHOLOGICAL ASSOCIATION (APA)

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Alexandra Blumhorst
Study Coordinator, Department of Psychiatry
August 3-5, 2023, Washington, DC

The American Psychological Association (APA) annual convention was held in Washington, D.C. and I was able to attend through my work at the Center for the Study of Traumatic Stress (CSTS) in the USU Department of Psychiatry. According to the APA, the APA convention is the largest meeting of psychologists, and this year's conference featured a diverse array of topics representing the wide range of interests of psychologists. I noted considerable emphasis on the theme of diversity, equity, and inclusion (DEI) throughout the conference, as well as discussions around ethics, COVID-19, mental health challenges related to current events, and the impact of technology on psychology.

Given CSTS's ongoing leadership in the DoD-funded Study to Assess Risk and Resilience in Servicemembers — Longitudinal Study (STARRS-LS), which seeks to provide information on risk reduction and resilience-building for suicide, suicide-related behavior, and other mental/behavioral health issues in the military, I was especially excited to attend a panel on military and veteran suicide featuring senior officials from the Departments of Defense and Veterans Affairs and expert researchers on suicide. Throughout the two-hour panel, common themes included the recognition that service members and veterans are especially vulnerable to suicide, suicide prevention in the military will require cultural and environmental changes, and suicide is a complex problem that requires a comprehensive approach.

With a small team of researchers from CSTS, I co-authored and virtually presented a poster analyzing the association between feelings of mattering and valuing COVID-19-related work and anger and alcohol use in National Guard (NG) service members. The poster was part of a larger study on a specific NG unit that activated early in the COVID-19 pandemic and discussed the utility of better understanding the connection between feelings of importance and value and anger and alcohol use in order to promote resilience in the NG.

This was my first-time attending APA and I really enjoyed exploring the multitude of events available. Over the three days, I attended a variety of symposia, critical conversations, and skill building workshops, as well as met with other professionals working in the field of psychology. I am eager to attend APA again in the future!

3 ACADEMY OF NUTRITION AND DIETETICS FOOD AND NUTRITION CONFERENCE AND EXPO (FNCE)

Dr. Jonathan Scott, Department of Military and Emergency Medicine
October 7-10, 2023, Denver, CO

Representing more than 112,000 credentialed nutrition and dietetics practitioners, the Academy of Nutrition and Dietetics (AND) is the world's largest organization of food and nutrition professionals. The Academy is committed to improving health and advancing the profession of dietetics through research, education and advocacy. At the AND 2023 Food & Nutrition Conference & Expo® (FNCE), registered dietitians, nutrition and dietetic technicians, registered, nutrition science researchers, policymakers, health care providers and industry leaders participated in more than 100 cutting-edge nutrition science research and education presentations, panel discussions and poster presentations. Attendees explored the latest advances in medical nutrition therapy, health care technology, and nutrition services access.. For students interested in nutrition, this conference provides a great mix of both research and practical application.

I had the opportunity to present a session entitled: "Dietary Supplements in the Military: Use, Threats, and Proposed Educational Solutions" with my colleagues from the Consortium for Health and Military Performance (CHAMP) Ms. Andrea Lindsey, Director Operation Supplement Safety (OPSS) and Senior Nutrition Scientist and Ms. Maria McConville, Senior Nutrition Health Educator. Several studies have consistently reported use dietary supplements in the military is ubquitous. Approximately 75% of all US Service Members self-report using at least one dietary supplement on a weekly basis. Service Members report using dietary supplements for performance enhancement and for optimizing mission success. Unfortunately, not all dietary supplements are health-promoting, and some may pose unintended risks of harm, threatening military readiness and ultimately one's career. Recently, the Department of Defense has issued a formal instruction on the use of dietary supplements (DoDI 6130.06). The instruction mandates: 1. education for all Service Members and providers, 2. adverse event reporting by providers, 3. establishes a list of prohibited ingredients, and 4. formally establishes Operation Supplement Safety as the Department Defense's "go-to" educational authority on dietary supplements. Operation Supplement Safety works closely with Federal partners and community partners to identify gaps in resources, and develops evidence-based educational solutions. Held annually in October, this conference brings together world-renowned nutrition and health experts.

4 PHAGOCYTES GORDON RESEARCH CONFERENCE (GRC)

Dr. Jeremy Rotty, Department of Biochemistry
June 4-9, 2023 in Waterville Valley, NH

I had the opportunity to attend the 2023 Phagocytes GRC in Waterville Valley, NH. Though technically summer, it felt more like early spring. I highly recommend that if you plan to attend a meeting in New England around the same time you don't pack as if you're headed to Myrtle Beach, like I did. Despite being cold the whole week, and stubbornly refusing to buy weather-appropriate clothing, I had a fantastic time!

The theme of the meeting was 'Molecular and Cellular Diversity in Host Defense and Inflammation'. It was chaired by Sergio Catz (Scripps Research Institute), and vice-chaired by Alison Criss (UVA). The first session was highly relevant to our lab, consisting of two keynote talks from Klaus Ley (Augusta University) on Beta2 integrin structure and function, and Ana-Maria Lennon-Dumenil (Institut Curie) on phagocyte cell shape changes and behavior under experimentally-applied confinement. There were many talks centered on neutrophil extracellular traps (NETs), the sticky DNA-containing extrusions that are thought to immobilize extracellular pathogens. In particular, talks by Ben Croker (UCSD), Luciane Dias-Melicio (Sao Paulo State University), and Arturo Zychlinsky (Max Planck Institute) helped shine a light on this topic. Matthew Lawrenz (University of Louisville) went medieval, taking us all the way back to the Black Death, explaining how Yersinia pestis alters neutrophil function.

Another theme of the week was the beautiful imaging and innovative approaches being leveraged to study phagocyte behavior. Sofia de Oliveira (Albert Einstein College of Medicine) presented work utilizing in vivo imaging of zebrafish to better understand dynamic neutrophil behavior. Gustavo Menezes (Federal University of Minas Gerais, Brazil) presented a highly engaging and expansive discussion of how to use intravital two-photon microscopy to image several different organs in live mice. Perhaps the most amazing was his ability to image myeloid cells in the neonatal mouse liver! Carole Parent (U. Michigan) reported remarkable findings on neutrophil chemotaxis, NET production, and how these cells produce LTB4-containing exosomes. Speaking of LTB4, Balázs Enyedi (Semmelweis University) developed novel biosensors to detect LTB4, a powerful neutrophil chemoattractant, in real time! Meghan Morrissey (UC Santa Barbara) presented her lab's work using DNA origami to understand phagocyte signaling, particularly during phagocytosis. Finally, Laurel Hind (UC Boulder) spoke about her lab's efforts to develop a microfluidic system to model multicellular interactions during innate immune response to infection.

Finally, there were an equal number of compelling talks on macrophage, microglia and dendritic cell function. One of the most engaging was Roberto Botelho's (Toronto Metropolitan University) talk on macrophage phagocytic exhaustion, or, how macrophages get full. Sergio Grinstein (Hospital for Sick Children, Toronto) gave a remarkable talk about how phagocytes detect phosphatidylserine (PS) during efferocytosis. Dorothy Schafer (UMass Medical School), Anna Victoria Molofsky (UCSF), and Amal Amer (Ohio State) all gave excellent talks on microglia encompassing topics including the mechanisms of synaptic pruning, microglia in brain development, and non-canonical inflammasome induction.

With apologies to all the speakers I can't incorporate due to space constraints, let me just say that there was far too much great science to cover in a succinct fashion! In closing, I also want to highlight a feature that the Gordon Research Conferences have adopted at every meeting: the GRC Power Hour. This is an optional discussion session meant to encourage discussions of diversity, inclusion and professional growth in an open, collegial setting. I did attend the session at the Phagocytes meeting and found it useful both as a young PI, and as a research mentor.

Each GRC is deliberately kept to a relatively small number of attendees, and all attendees are encouraged to be present the entire week. We all share common meals and go to the same talks and posters, so it is almost impossible to not bump into everyone at the meeting at least once. This facilitates networking tremendously, so even if you don't go to the Phagocytes meeting I highly encourage junior trainees to find a GRC that's right for them. Years later, I remain in contact with people I met at GRCs during graduate school and as postdoc. They have become collaborators, colleagues, and even friends. Just check the weather beforehand if you're headed to New England in early June.

ANGELMAN SYNDROME FOUNDATION/DUP15Q ALLIANCE RESEARCH SYMPOSIUM

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Dr. Laura Drebushenko, Department of Anatomy, Physiology and Genetics

July 25-26, 2023 Nashville, TN

This summer, I had the opportunity to travel to Nashville, TN to attend the Angelman Syndrome Foundation (ASF)/Dup15q Alliance Research Symposium. Angelman Syndrome (AS) and Dup15q Syndrome are debilitating neurodevelopmental disorders that arise from mutations in maternal chromosome 15q11.2-13.1. Both disorders present similarly to autism and include severe cognitive and language impairments, motor ataxia, and seizures. Because of the similarities between these two disorders, clinicians and researchers studying either or both gather each summer to discuss research progress. The research portion of the event on July 25-26th was a small, single-track conference featuring unpublished data. It was followed by a three-day conference for families of Dup15q patients.

Our lab was fortunate to have my thesis work on identifying dysregulated proteins in AS funded by ASF for the past two years. We were invited to share our progress at this summer's meeting, so I had the privilege of presenting my thesis work to a room full of experts in the field! Although nerve-wracking, this small community of researchers has been extremely welcoming and helpful to us as newcomers to the field, and I thoroughly appreciated the opportunity to meet many of those whose work I have been following for the past six years. In addition to the basic research that many presented, there were also updates on larger-scale human studies and databases. For example, the Angelman Syndrome Natural History Study is a multicenter longitudinal study that began in 2006, which aims to analyze the behavior, communication, and development of AS patients throughout their lives. Another critical effort in the study of these disorders is LADDER, a database of both AS and Dup15q patient data from clinics and research studies around the world that can be accessed and used by researchers. There were also non-scientific members of these organizations in attendance who shared their efforts to increase awareness and provide us with as many tools as possible for use in our studies.

A unique benefit of a small, narrowly focused meeting like this one is that our common goal of improving the lives of those affected by AS and Dup15q creates a strong sense of community and collaboration. Clinicians in the audience were eager to take back to the clinic what they learned from basic research studies indicating that other therapeutics might ameliorate AS symptoms better than the first line treatments typically prescribed. On the other hand, researchers wanted to hear complaints from families and clinicians so they could address these issues in their studies. Veterans in the field even provided me with some feedback and new ideas to include in my own work, such as some alternate developmental timepoints to explore, which I have since incorporated into my project. Overall, it was a wonderful experience.

Although this particular conference might not be of direct interest to many others at USU, I do highly recommend that anyone else who studies rare conditions dive deeper into small, tight-knit conferences like this one to provide a more personal impact like ASF/Dup15q did for me.

6 EUROPEAN MOLECULAR BIOLOGY LABORATORY (EMBL): PROTEIN SYNTHESIS AND TRANSLATIONAL CONTROL

Megan Rasmussen, MCB PhD Candidate
September 6 – 10, 2023 Heidelberg, Germany

The European Molecular Biology Laboratory (EMBL) 2023 Protein Synthesis and Translational Control conference was my first academic conference experience as a graduate student. Although I attended virtually, it was amazing to see the enthusiasm and curiosity of those in the audience. For five days, several hundred scientists flocked to Germany, excited to learn and discuss a wide range of topics related to protein synthesis, including not only the mechanisms of translation, but also its implications in human disease and organismal biology.

A fascinating talk focused on ribosomal protein paralogs and their role in disease was given by Katarina Grobicki, a postgraduate student in Dr. Felipe Teixeira's lab at the University of Cambridge. Several groups have suggested that ribosomes are heterogeneous and exist in pools containing differential post-translational modifications, accessory proteins, and even ribosomal protein paralogs that may affect ribosome speed, fidelity, or transcript selection for translation. These so-called "specialized ribosomes" are of considerable interest as mutations in the ribosomal machinery and biogenesis factors cause distinct disease phenotypes that could be driven by defects in the function of specific ribosome pools. Grobicki and colleagues addressed the role of alternative ribosomal protein paralogs in ribosome function using Drosophila melanogaster as their model (with 19 unique ribosomal protein paralogs at their disposal). Intriguingly, they found that most ribosomal protein paralogs are not required for viability or fertility when individually knocked out, suggesting functional redundancy between alternative paralogs. However, ribosomal subunit protein 5b (RpS5b) was required within the female germline for oocyte development, while it's paralog RpS5a was not. The investigators found that RpS5a is ubiquitously expressed in most cells during development, excluding some subtypes like the germ cells of the ovariole. In contrast, RpS5b expression was restricted to the germ cells during oogenesis. Knockout of the RpS5b paralog resulted in egg chamber death at midoogenesis and total sterility. To explore the possibility of specialized ribosomes containing either RpS5a or RpS5b, the investigators replaced the RpS5b protein coding sequence with that of RpS5a. RpS5a expression in germ cells during oogenesis rescued the Rps5b knockout oocyte development defect, leading Grobicki et al. to conclude that the developmental phenotype was due to a decrease in ribosomal biogenesis and overall protein synthesis rather than a loss of a specialized Rps5b-containing ribosome pool. This important work highlighted that while ribosomal protein paralogs are functionally redundant in Drosophila melanogaster, specific cell types and tissues may be reliant on one paralog more than the other based on paralog expression patterns.

I recommend this conference to anyone at USUHS who wants to learn more about gene expression. Protein synthesis dysregulation is implicated in an increasing number of diseases, emphasizing the importance of translation regulation on protein levels and cell function. This conference was incredibly eye opening and I loved seeing the speakers' dedication toward their work and passion for sharing knowledge with an equally enthusiastic audience.

INDO-PACIFIC MILITARY HEALTH EXCHANGE

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Sharon Kim, MD/PhD Student, Class of 2026
September 26-29, 2023 Kuala Lumpur, Malaysia

This past September, I had the privilege of attending the 2023 Indo-Pacific Military Health Exchange (IPMHE) in Kuala Lumpur, Malaysia. IPMHE is funded through the USU Center of Global Health Engagement (CGHE) and facilitated by Dr. Tamara Worlton, who is the faculty adviser for the USU Global Surgery Interest Group. IPMHE is a multilateral military event focused on partnership and interoperability and is co-hosted by the armed forces of a country in the Indo-Pacific region together with the United States Indo-Pacific Command, or USINDOPACOM, Command Surgeon. The co-host for IPMHE 2023 was the Malaysian Armed Forces Health Services. At the conference, I presented my poster titled, "Promoting Global Surgery and Anesthesia (GSA) as Essential Curriculum for Uniformed Medical Students," which has also been published in the Journal of Military Medicine.1 My poster presentation emphasized the critical role of GSA education at USU in preparing the next generation of uniformed physicians for global surgical missions and addressing disparities in surgical care access worldwide.

During the poster session, I found that military leaders from diverse nations were genuinely captivated by the burgeoning field of global surgery and the growing interest among medical students to actively engage in it. Notably, a military physician from Japan's military medical school highlighted a concerning trend – a decline in student interest in surgery overall. This observation was echoed by counterparts from South Korea, underscoring a similar decline in their country over the years. This sparked my curiosity about the reasons behind this trend, leading to insightful discussions on the impact of exposure, education, and health systems on shaping students' interest in the field. There were also discussions on the implications of such trends on military surgical readiness, a pivotal element in trauma care. As a military medical student, I advocated for global surgery awareness through platforms like the International Student Surgical Network (IncisioN), akin to the Global Surgery Student Alliance (GSSA) in the US.

I was also able to gain valuable insights from the conference's various speakers and activities. One of the keynote speakers, Major General Paula Lodi, emphasized the critical role of sustainability in military medicine and the often-limiting factor posed by changes in leadership. This resonated with me, reinforcing the relevance of my poster. I believe that education plays a pivotal role in nurturing the next generation of leaders, fostering advancements at both individual and systems levels. Further, a key takeaway from the global surgery panel was the recognition that global surgery is not exclusive to surgeons; rather, it constitutes a collective effort encompassing all healthcare workers, particularly at the systems level. Finally, a site visit with Malaysia's military medical counterparts left a lasting impression. I toured a large Role 3 field hospital with diverse medical capabilities, ranging from radiological, surgical, ophthalmologic, to even dental services. From this experience, I realized the value for USU students to engage in exchange educational experiences at other military medical schools, similar to how international students visit USU through various initiatives, including Operation Bushmaster.

IPMHE 2023 reinforced that military medicine is a collaborative global effort, transcending borders and fostering a collective desire for improvement. The exchange of ideas and experiences showcased the eagerness of military healthcare personnel worldwide to learn from one another, highlighting the importance of collaboration in advancing military medicine. And beyond its role as a hub for knowledge exchange, IPMHE was a platform for cultural engagement. For instance, witnessing top leaders shed their official military uniforms to connect on a personal level with friends and family at the cultural dinner night was a testament to the essence of true health engagement. The conference demonstrated the importance of relationship-building, sustaining existing partnerships, as well as bridging cultural and political differences.

In conclusion, IPMHE 2023 was a phenomenal educational experience. I had the opportunity to advocate for military global surgical education and gain firsthand insights into how global health engagement operates in the military. The event highlighted the critical role of knowledge and cultural exchange in relationship-building and forging collaborations while sustaining existing ones. Military medicine, as supported by the global interest at IPMHE, is a collective endeavor, and events like these serve as opportunities for collective progress. As we navigate the ever-evolving landscape of military medicine, I want to emphasize the importance for students to immerse themselves in experiences that go beyond the classroom. Experiences such as IPMHE 2023 provide a tangible understanding of the field and foster genuine interest through exposure, connections with colleagues, and mentorship.

The American Public Health Association's Annual Meeting and Expo is an opportunity for public health professionals to gather to share the latest research and information regarding public health topics, and also promote and advocate for those public health issues that are supported by research. The premise of the annual conference is to collaborate, learn, and engage on a number of emerging and pertinent public health topics facing our nation today.

I was fortunate to attend this conference last year (2022) and present a poster on Operation Supplement Safety (OPSS). This year I presented a roundtable, along with my co-authors from University of North Carolina, Chapel Hill, Dr. Kim Faurot and Dr. Amanda Corbett. We were accepted under the Integrative, Complementary, and Traditional Health Practices (ICTHP) group, and presented on "How to use a screening tool to assess risk of dietary supplement" in the session, "Health and Wellness across the lifespan through ICTHP." We demonstrated how to use the OPSS Scorecard (see it at (https://www.opss.org/opss-scorecard-check-your-dietary-supplement) as an interactive educational tool to assess risk as well as discuss the importance of the individual screening questions. Attendees shared their experiences with dietary supplements and shared ideas to improve the dietary supplement safety environment for the health of the nation. We brought actual dietary supplements to the roundtable, and were able to "score" the products, both individually and as a group, and then discuss our answers together.

I would recommend this conference to any student in the public health space. This has now become my favorite conference, and I'm already planning my abstract to present again at next year's conference. The attendees and presenters are the ones doing the work on the ground and impacting state and local communities. I found the experience this year so gratifying as I had many providers attend the roundtable and the discussions were very helpful to my work.

8 AMERICAN PUBLIC HEALTH ASSOCIATION ANNUAL MEETING

Andrea Lindsey Director and Senior Nutrition Scientist, CHAMP
November 12-15, 2023 Atlanta, GA

NEUROSCIENCE 2023

Dr. Dylan Scarton, Department of Physical Medicine and Rehabilitation
November 11-15, 2023 Washington, DC

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I attended the 51st annual meeting of the Society for Neuroscience (SfN) founded in 1969. SfN boasts over 30,000 active members from nearly 100 different countries around the world. It supports the neuroscience community by advancing scientific exchange through engaging and educating the public and advocating for the field at large. Their regular programming includes publishing two highly regarded scientific journals (JNeurosci and eNeuro), offering professional development resources and career training through Neuronline, and organizing a variety of engaging public outreach efforts like expanding the interactive collection of public-facing resources on BrainFacts.org.

This year's annual meeting, Neuroscience 2023, was a unique one for me. Although I have attended all but one annual meeting since 2015, my participation this year was actually about my policy work instead of my research activities. As an Early Career Policy Ambassador (ECPA) through SfN Advocacy, I was invited to present a poster at a dedicated session on the evening of the first day to share about my science advocacy and public policy activities during my time in the program. These activities included the annual Capitol Hill Day and Summer Congressional Days events, as well as my planned engagement efforts involving community outreach with local politicians and students.

Overall, I really enjoyed the opportunity to meet my ECPA scholars in person and discuss the program with others who were interested in joining a future cohort. It was a very different experience relative to what I had come to expect from the typical research conference, which I found to be an especially enlightening and rewarding perspective. I would strongly encourage all of my USU colleagues to seek out this type of content at their upcoming meetings to see another side of science!

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AMERICAN SOCIETY FOR TROPICAL MEDICINE AND HYGIENE (ASTMH)

Joshua Trowell, Preventive Medicine Biostatistics PhD Candidate
October 18- 23, 2023 Chicago, IL

The American Society for Tropical Medicine and Hygiene (ASTMH) conference was held in Chicago this year and is the largest international scientific organization of experts dedicated to reducing the worldwide burden of tropical infectious disease to improve global health. This was my first time attending a conference with over 4,500 attendees from over 119 represented countries. I attended the conference to present my abstract, which is a systematic review of nifurtimox and benzimidazole used in randomized controlled trials (RCTs) to treat Trypanosoma cruzi, the parasite that causes Chagas disease. This conference exposed me to many fellow research scientist in the field that work in parallel to my research interests from all over the world.

The opening keynote speaker was Ambassador John Nkengasong, PhD, who is the Ambassador-at-Large, U.S. Global AIDS Coordinator, and Senior Bureau Official for Global Health Security and Diplomacy who reports directly to the U.S. Secretary of State, Antony Blinken. I was mesmerized by his ability to deliver an impactful message structured around four Ps for global health change: Policies, Partnerships, Pathogens, and Population, which are integral to improve population health. His inspirational message and impact was uniquely woven into his experiences guiding Africa through the COVID-19 pandemic as Director of Africa CDC, and his current work with President's Emergency Plan for AIDS Relief (PEPFAR).

After presenting my work to fellow conference attendees, I was able to network with members that were interested in Chagas disease research, specifically, scientists that worked closely with vulnerable populations in Central and South America. Since Chagas disease is mostly endemic in the Americas, I received a lot of feedback for my poster include RCTs conducted in other countries, as studies most likely written in Spanish or Portuguese could offer addition insight into my research question.

I would recommend this conference to current graduate students and faculty members interested in global health and neglected tropical diseases (NTDs). My first conference as a graduate student was a huge success, and I am eager to attend the next annual meeting in November 2024 that will take place in New Orleans. My future research goals entail understanding and elucidating the landscape of Chagas disease among foreign born military service members, in a descriptive epidemiological analysis to map disease burden and model future disease risk in this population. After this experience, my goal is to continue to learn from the many accomplished researchers and scientists in this global field.

We welcome any inquiries or feedback
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