NUS National University of Singapore

Department of Microbiology and Immunology
Yong Loo Lin School of Medicine

2025 CENTENNIAL SYMPOSIUM

A Century of Discovery: Advancing Microbiology and Immunology

SINCE 1925

MICROBIOLOGY & IMMUNOLOGY 100

Celebrating a Legacy of Academic Excellence

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A Century of Discovery: Advancing Microbiology and Immunology

Guest of Honour
Professor Kenneth Mak
Director-General of Health, Ministry of Health Singapore

Dean, NUS Yong Loo Lin School of Medicine
Professor Chong Yap Seng
Lien Ying Chow Professor in Medicine

HOD, Department of Microbiology and Immunology
Associate Professor Kevin SW Tan
Dean's Chair

Programme Co-Chairs
Associate Professor Justin Chu Jang Hann
Associate Professor Zhang Yongliang

Special thanks to our Organizing Committee:

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Dean's Address

Chong Yap Seng
Lien Ying Chow Professor in Medicine
Dean, NUS Yong Loo Lin School of Medicine

There are sound reasons why we constantly exhort our students to build a solid foundation in microbiological and immunological concepts such as advances in molecular biology, antimicrobial therapy and vaccine development, and then learn to apply these to clinical practice.

Think of Covid-19 and the earlier SARS pandemics that rattled the world. Both outbreaks severely tested our healthcare system. We emerged from both crises wiser and better informed because intrepid microbiological and immunological scientists in Singapore worked hard to find solutions, in collaboration with their colleagues around the world.

The British poet William Blake's poem, Auguries of Innocence, starts with this stanza:

To see a world in a grain of sand
And a heaven in a wild flower,
Hold infinity in the palm of your hand
And eternity in an hour.

Through closely examining something very minute, one may actually be able to glimpse and appreciate the profound vastness and interconnectedness of our universe. I like to think that it is this philosophical mindset that may have inspired the work of the academic staff at the Departments of Bacteriology and Parasitology (antecedents of the Department of Microbiology and Immunology at the National University Singapore) in 1925. An early chair of the department was Professor Nripendra Kumar Sen: his work on brucellosis in Singapore's then numerous cattle gave the island its own eponymous pathogen, i.e., Salmonella Singapore (now S. enterica serovar Singapore)[1].

Blake's lines continue to fire the imagination of the colleagues at the Department in our time: consider the groundbreaking work of Professor Lim Kok Ann, who was the Dean of the then Faculty of Medicine from 1965 to 1972 and the first Singaporean Professor of Bacteriology and Head of Department between 1959 and 1977. He rose to international prominence for his work in isolating the flu virus during the Asian influenza epidemic, and is also remembered for his pioneering work in introducing the Oral Sabin Polio Vaccine to eradicate poliomyelitis in Singapore children.

Consider also the legacy of the late Emeritus Professor Chan Soh Ha, another titan of the department. He won the National Science and Technology Award in 1992 for the discovery of a new tissue type antigen called Singapore-2 (HLA-B46) which is associated with people of Chinese descent. The antigen is associated with diseases such as nasopharyngeal carcinoma, rheumatoid arthritis, and Grave's disease. He also served as Director of the WHO Immunology & Training Centre from 1976 to 2006 and helped establish Singapore as a leading centre for tissue typing in the Asian region. Colleagues remember him as a visionary who saw the importance of the Asian phenotype, years before it was explored in earnest. Prof Chan is also credited for contributing his scientific expertise to helping the establishment of the National University Medical Institute (NUMI), Cancer Science Institute (CSI) and Cardiovascular Research Institute (CVRI).

Years, decades and a century on, the pursuit of scientific excellence at the Department is relentless, with on-going work on infectious disease a signature endeavour that sees investigators collaborating with clinical colleagues and other experts under a unified NUS Medicine translational research programme. Along the way, the Department has produced its fair share of academic leaders such as Professor Mary Ng, current department head Associate Professor Kevin Tan, Associate Professor Paul MaCary, Associate Professor Justin Chu and Associate Professor Veronique Angeli.

Even as the NUS Department of Microbiology and Immunology looks back with quiet pride on a century of achievement, it must keep its gaze on the future and continue to sail uncharted seas in the never-ending quest for scientific truth and understanding, while tending faithfully to the education of future generations of healthcare and science professionals. It is discovery and teaching work that continues to exert a qualitative contribution to the health and well-being of Singaporeans and people everywhere.

Happy 100th anniversary! Here's to many more years of inspiring health together.

Prof Chong Yap Seng
Lien Ying Chow Professor in Medicine
Dean, NUS Yong Loo Lin School of Medicine

[1] The Evolution of Teaching and Learning Medical Microbiology and Infectious Diseases at NUS MB Taylor, VTK Chow, Annals Academy of Medicine, July 2005, Vol. 34, No. 6

HoD's Address

Kevin SW Tan
Associate Professor
Head of Department,
Department of Microbiology and Immunology

Dear Esteemed Guests, Colleagues, and Students,

It gives me great pleasure to welcome you to the centennial symposium of the Department of Microbiology and Immunology at the National University of Singapore, themed "A Century of Discovery: Advancing Microbiology and Immunology." We are privileged to have with us today our Guest of Honor, Professor Kenneth Mak, Director-General of Health at the Ministry of Health, Singapore; Professor Chong Yap Seng, Dean of the Yong Loo Lin School of Medicine; and Professor Yeoh Kay Guan, Chief Executive of the National University Health System.

Since its inception in 1925, the Department has evolved significantly, rooted in the historical Departments of Bacteriology and Parasitology. Our department has been at the forefront of medical education and research, addressing the challenges of infectious diseases and advancing our understanding of immunology and host-microbe interactions.

The department's remarkable progress and reputation today are the result of the strong foundations of research and teaching excellence established by generations of predecessors. The dedication and vision of academic staff, laboratory technologists, administrative colleagues, and not least, our graduate students—whose passion for discovery and learning continues to drive the department forward—have played an instrumental role in nurturing our growth and shaping our legacy. We stand on their collective shoulders as we continue our mission into the next century.

This symposium not only celebrates our illustrious past but also highlights our recent achievements and ongoing commitment to research and educational excellence. We are excited to welcome a distinguished lineup of speakers, including international experts such as Professor Malik Peiris from The University of Hong Kong, Professor Tadatsugu Taniguchi from The University of Tokyo, Professor Gao Fu from The Institute of Microbiology of the Chinese Academy of Sciences, and Professor James McCluskey from The University of Melbourne. Their talks will lend invaluable insights into the latest advancements in the fields of emerging diseases, pandemics and immune responses, greatly enriching our discussions and expanding our horizons.

In addition to our esteemed overseas speakers, we are proud to feature local experts including Professor Lisa Ng, Associate Professor Veronique Angeli, and alumni speakers Dr. Tan Hai Meng and Professor Lok Shee Mei, who will share their experiences and innovations in microbiology and immunology. The blend of international and local perspectives will foster engaging dialogue, encourage collaboration, and spark the exchange of new ideas.

A further highlight of this event will be the poster sessions, which showcase the innovative work of our graduate students and postdoctoral researchers. These sessions provide a unique opportunity for networking and interaction, allowing attendees to engage directly with the next generation of researchers who are pushing the boundaries of microbiological and immunological sciences.

As we commemorate this milestone, I invite all attendees to participate actively in the sessions, engage with our speakers, and explore the creative research presented by our students. Together, let us honor our legacy and continue to explore new frontiers in microbiology and immunology.

Thank you for being here to celebrate this momentous occasion with us. I look forward to a day filled with inspiring discussions and fruitful networking.

Warm regards,

A/Prof Kevin SW Tan
Head of Department
Department of Microbiology and Immunology
National University of Singapore

CONTENT

Programme Schedule

Venue: MD11 Clinical Research Centre (CRC), 10 Medical Drive, Yong Loo Lin School of Medicine, Singapore 117597

Date: 18th July 2025

0800-0845 **Registration**

0845-0915 **Welcome and Opening Address (*Master of Ceremony: Dr Png Chin Wen, NUS*)**

- ***Professor Chong Yap Seng***, *Dean of the Yong Loo Lin School of Medicine, NUS*
- ***Associate Professor Kevin Tan***, *Head of the Department of Microbiology and Immunology, NUS*
- *Guest of Honour:* ***Professor Kenneth Mak***, *Director-General of Health, MOH Singapore*

Session 1 ***Session Moderator: Associate Professor Sylvie Alonso, NUS***

0915-1000 **Keynote Address 1:** Avian flu H5N1 saga: past, present, future
Professor Malik Peiris, *The University of Hong Kong*

1000-1030 **Invited Alumni Talk:** From bench to boardroom: my journey through life as a microbiologist
Dr. Tan Hai Meng, *Kemin Industries Inc.*

1030-1100 Coffee Break

Session 2 ***Session Moderator: Associate Professor Zhang Yongliang, NUS***

1100-1145 **Keynote Address 2:** Interferons and beyond; what could be the struggle to fill our heart?
Professor Tadatsugu Taniguchi, *The University of Tokyo*

1145-1215 **Invited Talk:** Viral warfare frontiers in cellular mechanisms and therapeutic interventions
Professor Lisa Ng, *A*STAR*

1215-1345 Lunch Break and Poster Viewing Session

Session 3 ***Session Moderator: Associate Professor Vincent Chow, NUS***

1345-1430 **Keynote Address 3:** COVID-19: virus and receptor discovery and development of vaccine and antibody
Professor Gao Fu, *The Institute of Microbiology of the Chinese Academy of Sciences*

1430-1500 **Invited Talk:** Breakthrough discovery of Lyve-1+ tissue resident macrophage functions through lymphatic research
Associate Professor Veronique Angeli, *NUS*

1500-1530 Coffee Break

Session 4 ***Session Moderator: Associate Professor Tan Yee Joo, NUS***

1530-1615 **Keynote Address 4:** MR1-restricted immunity and the emerging importance of MAIT cells in health and disease
Professor James McCluskey, *The University of Melbourne*

Programme Schedule

Venue: MD11 Clinical Research Centre (CRC), 10 Medical Drive, Yong Loo Lin School of Medicine, Singapore 117597

Date: 18th July 2025

1615-1645 **Invited Alumni Talk:** Tomography studies of dengue fusion process
Professor Lok Shee Mei, *Duke-NUS*

1645-1700 **Prize Presentation and Closing Address**

- ***Associate Professor Kevin Tan***, *Head of the Department of Microbiology and Immunology, NUS*

KEYNOTE SPEAKER

PROFESSOR MALIK PEIRIS

THE UNIVERSITY OF HONG KONG

SPEAKER'S PROFILE

Professor Malik Peiris is currently Professor of Virology at the School of Public Health at The University of Hong Kong. He is a clinical and public health virologist, with an interest in emerging virus disease at the animal-human interface, including vector-borne viruses, influenza and coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2). In 2003, he contributed to identifying the novel coronavirus that caused SARS and to understanding its origins, diagnosis and control. More recently, he works on zoonotic, seasonal and pandemic influenza, MERS and COVID-19 using a One Health approach. He is elected as a Fellow of the Royal Society of London and as a Foreign Member of the US National Academy of Sciences.

INVITED ALUMNI SPEAKER

DR. TAN HAI MENG

KEMIN INDUSTRIES INC.

SPEAKER'S PROFILE

Dr. Tan Hai Meng is the President of Kemin Animal Nutrition and Health – Asia Pacific, where he has dedicated 25 years of exceptional service. His journey at Kemin began as Vice-President of Research & Development, followed by his promotion to Senior Vice-President of Sales, and ultimately rising to his current role in 2016. Throughout his tenure, Dr. Tan has made significant contributions to Kemin, earning numerous prestigious awards for his outstanding achievements. Among his accolades, he was honored with the R.W. Nelson Medal for his innovative patented products such as KEMZYME Multi-Protease™, Myco CURB® Extend, Quantum GLO™, and CLOSTAT™. Notably, CLOSTAT® achieved remarkable sales exceeding US$ 250 million. Dr. Tan and his team are credited with inventing over 12 patents, leading to his recognition as Kemin Worldwide Outstanding Executive of the Year. Under his visionary leadership, Kemin Asia has garnered accolades from various professional bodies, including being named one of the Best Companies to Work for in Asia by HR Asia (2017) and winning the Best Talent Management Practices Award by the Singapore Institute of Management (2018). Dr. Tan's exemplary leadership was further acknowledged when he was conferred Leading CEO at the Singapore HR Awards (2019) and recognized as Top CEO (Agriculture) by Singapore Business Reviews last year. Dr. Tan holds a Ph.D. in Microbiology from King's College in London, UK. Before joining Kemin, he was a tenured Associate Professor of Microbiology at the National University of Singapore and an Alexander von Humboldt Foundation Visiting Scholar at the German Research Centre for Biotechnology (GBF) in Braunschweig, Germany. Beyond his professional achievements, Dr. Tan has actively contributed to various boards and committees, including the Singapore Science Centre Board, the Nanyang Polytechnic Food Science Committee, and NGO boards such as the Lakeside Family Centre Management Committee and CRU. He was appointed a Distinguished Professional at the Republic Polytechnic and currently serves on the National Research Project Evaluation Panel of the Singapore Food Agency.

KEYNOTE SPEAKER

PROFESSOR TADATSUGU TANIGUCHI

THE UNIVERSITY OF TOKYO

SPEAKER'S PROFILE

Professor Tadatsugu Taniguchi's pioneering research began with the discovery and molecular characterization of two key cytokine genes: human fibroblast interferon (later renamed IFN-β) and interleukin-2 (IL-2). His work laid the foundation for the large-scale production of these cytokines for clinical applications—IFN-β for viral infections and multiple sclerosis, and IL-2 for cancer immunotherapy. While investigating the mechanisms underlying cytokine gene expression and signaling, his laboratory identified a novel family of transcription factors known as interferon regulatory factors (IRFs). This family has since emerged as a central player in the regulation of the immune system and cancer biology. Professor Taniguchi's more recent research has led to the discovery of DNA sensing molecules such as DAI (DNA-dependent activator of IRFs) and HMGB proteins, which activate innate and adaptive immune responses through IRFs and other transcription factors. His current work focuses on understanding how molecules derived from dead cells contribute to immune disorders and anti-tumor immunity, as well as exploring their therapeutic potential—particularly in cancer—through antibodies and related approaches. His prolific research output includes 57 publications in top-tier journals such as Nature, Science, and Cell.

INVITED SPEAKER

PROFESSOR LISA NG

A*STAR

SPEAKER'S PROFILE

Professor Lisa Ng currently serves as Executive Director at the A*STAR Infectious Diseases Labs and holds a joint appointment at the Biomedical Research Council, A*STAR. With over 25 years of expertise in infectious diseases, she has made significant contributions to the containment, prevention, and treatment of epidemic viral infections such as SARS and avian influenza H5N1. Her current research focuses on immune responses to epidemic and endemic viruses in tropical regions, including chikungunya, dengue, Zika, and SARS-CoV-2. Her team's findings, published in leading scientific journals, have advanced human immunology in viral infection control. Recognized as 'Most Inspiring Woman' at the Great Women of Our Time Awards (2005) and recipient of the Junior Chamber International 'Ten Outstanding Young Persons of the World' Singapore Award (2013), she also received the ASEAN 'International Young Scientist and Technologist Award' (2008) for her research on Asia's infectious diseases. Dr. Ng's dedication to mentoring has earned her the A*STAR 'Most Inspiring Mentor Award' (2013). Her accolades include the Public Administration Medal (Bronze, 2016), National COVID-19 Award (Silver, 2022), and Public Administration Medal (Silver, 2023). Recognized as a SNAS fellow (2022) and an elected member of the Henry Kunkel Society for immunologists, she is listed among the Top 2% Scientists Worldwide and Highly Cited Researchers for 2023 and 2024.

KEYNOTE ADDRESS

PROFESSOR GAO FU

THE INSTITUTE OF MICROBIOLOGY OF THE CHINESE ACADEMY OF SCIENCES

SPEAKER'S PROFILE

Professor Gao Fu is a distinguished virologist and public health leader who earned his doctorate from the University of Oxford and conducted postdoctoral research at Oxford, Harvard, and the University of Calgary. He previously served as director-general of the Chinese Center for Disease Control and Prevention (China CDC) and vice-president of the National Natural Science Foundation of China (NSFC). Currently, he is a professor at the Institute of Microbiology, Chinese Academy of Sciences and China CDC. A pioneer in virology and immunology, Dr. Gao has made groundbreaking contributions to understanding the interspecies transmission of influenza and coronaviruses. His research has significantly advanced the control of emerging infectious diseases, with high-impact publications in Nature, Science, Cell, The Lancet, and The New England Journal of Medicine (H-index: 142). During the COVID-19 pandemic, he co-discovered SARS-CoV-2 and led the development of the world's first approved neutralizing antibody therapy (Etesevimab) and recombinant protein vaccine (ZF2001). An elected member of multiple academies—including the Chinese Academy of Sciences (CAS), U.S. National Academy of Sciences (NAS), and UK Royal Society—he has received honors such as the TWAS Award, Nikkei Asia Prize, and Gamaleya Medal. Dr. Gao also champions science communication as editor-in-chief of Science Bulletin and Chinese Science Bulletin (In Chinese), and founding editor of China CDC Weekly and hLife.

INVITED SPEAKER

A/PROFESSOR VERONIQUE ANGELI

NATIONAL UNIVERSITY OF SINGAPORE

SPEAKER'S PROFILE

A/Professor Veronique Angeli has been the leader of the Immunology Programme at Life Sciences Institute, NUS since July 2018 and the Director of Immunology Translational Research Programme at NUS Yong Loo Lin School of Medicine since 2020. She received her PhD in 2001 from the University of Lille in France. After her post-doctoral training at Mount Sinai School of Medicine in New York city, USA from 2002 to 2005, she joined the Department of Microbiology and Immunology at the National University of Singapore Yong Loo Lin School of Medicine as an Assistant Professor, and was promoted to Associate Professor in 2014. As a recognition of her research contributions, she received the Faculty Young Investigator Award in 2011, the Mochtar Riady Pinnacle Young Achiever Award from National University of Singapore in 2015, and Research Excellence Award from Yong Loo Lin School of Medicine in 2022. Her laboratory has advanced our understanding on the functions of lymphatic vessels in immunity, inflammation and lipid transport and published seminal work in top journals including Blood, Cell Metabolism and Immunity. She is now exploiting some of this knowledge to design new therapeutic strategies for lymphatic diseases including lymphedema. As a prominent lymphedema researcher, Professor Angeli is actively involved in bringing awareness about lymphedema, a debilitating condition particularly affecting cancer survivors, in Singapore and around the world. More recently, Professor Angeli's team uncovered a unique population of tissue- resident macrophage expressing Lyve-1 capable of controlling tissue collagen deposition at steady state. The contribution of this macrophage in vascular diseases, ageing and is ongoing with the promise to open the ways to new therapeutic strategies for fibrosis, wounds and tissue ageing.

KEYNOTE ADDRESS

PROFESSOR JAMES MCCLUSKEY

THE UNIVERSITY OF MELBOURNE

SPEAKER'S PROFILE

Professor James McCluskey is Assistant Vice Chancellor at the University of Melbourne following a period as DeputyVice-Chancellor (Research) between 2011-2023. In 2021 he acted as Provost of the University. He trained in Perth asa physician and pathologist before embarking on a life of research into the workings of the immune system, initially at the US National Institutes of Health and later at Monash University, Flinders University and since 1997 at The University of Melbourne. He has won a number of prizes for his research and has been a Clarivate Highly Cited researcher in Immunology since 2017. His research has focused on immune recognition, autoimmunity and antigen presentation. More recently, his work has opened up the field pf Mucosal Associated Invariant T cells (MAIT cells) and their roles in protective immunity, wound healing, cancer, autoimmunity and immunopathology. He has served as a board director of multiple independent medical research institutes including WEHI, Florey, Burnet, St Vincent's Institute and the Victorian Comprehensive Cancer Centre Alliance. He is a foundation board member of the AtlanticInstitute (Oxford). He is also a director of the Australian Friends of ASHA (a charity to improve life in Indian slums) and Trinity College Melbourne. He established the South Australian node of the Australian Bone Marrow Donor Registry and for over 30 years has been a consultant immunologist in transplantation matching for Life Blood, the Australian Red Cross Blood Service. He led the development of the Peter Doherty Institute for Infection and Immunity and co-chairs the Steering committee of the $700M Australian Institute for Infectious Disease. He also led the team that won $80M in grants from The Atlantic Philanthropies to help to establish the Atlantic Fellows for Social Equity program focused on indigenous leadership to effect social change. He is a Fellow of both the Australian Academy of Science and Academy of the Australian Academy of Health and Medical Science. He became an Officer of the Order of Australia (AO) in 2018. Jim wasawarded The prestigious Melbourne Achiever Award. The award recognises Professor McCluskey's extensiveindividual contributions to the biomedical research sector and the community in a career spanning nearly 30 years.

INVITED ALUMNI SPEAKER

PROFESSOR LOK SHEE MEI

DUKE-NUS

SPEAKER'S PROFILE

Professor Shee-Mei Lok is a Provost's Chair Professor in the Emerging Infectious program in the Duke-NUS, Singapore. She was also a National Research Foundation (NRF) fellow (2009-2014) and a NRF Investigator (2016-2021). She is a structural virologist specializing in x-ray crystallography and cryo-electron microscopy. Her research interest focuses on the structural and therapeutics aspects of flavivirus such as dengue and zika viruses. She obtained her Msc and PhD in NUS and did her post-doctoral training in Purdue University under the supervision of the late Hanley Prof Michael Rossmann. Her laboratory made significant discoveries in the morphological diversity of dengue virus particles, neutralization mechanisms of potent antibodies against flavivirus particles, the flavivirus assembly process and also the structures of the secreted dengue NS1 – an important factor that causes severe dengue disease.

Virology

Poster 1

Is a Nipah virus-specific vaccine sufficient to prevent the next henipavirus outbreak?

Wee Chee Yap[1], Beng Lee Lim[2], Madeline Sheng Si Kwek[2], Wan Ni Chia[2,3], Yun Yan Mah[2], Feng Zhu[2], Chee Wah Tan[1,2]

1 Infectious Disease Translational Research Programme, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, NUS, Singapore
2 Programme in Emerging Infectious Disease, Duke-NUS Medical School, Singapore
3 Leyden Labs Asia, Singapore

Surveillance of zoonotic viruses in wildlife has revealed that bats harbor multiple zoonotic pathogens, including the Nipah virus. Bat borne paramyxoviruses, especially henipaviruses pose a high risk of zoonotic spillover due to their use of highly conserved molecules as receptors. Preexisting human adaptive immunity, such as neutralizing antibodies, plays a crucial role in preventing zoonotic spillovers from becoming pandemics. This study aims to establish a high-throughput serological platform for the rapid detection of neutralizing antibodies against henipaviruses, facilitating disease risk assessment for known henipaviruses. We have established a surrogate virus neutralization assay that allows rapid detection of neutralizing antibodies that block the binding of the henipaviruses glycoprotein to the receptor in a single-tube reaction. With this platform, we perform a disease risk assessment of known henipaviruses by characterizing the cross-reactivity neutralizing antibodies of convalescent serum samples collected from Nipah patients. Besides, we characterize the cross-reactivity neutralizing antibodies of the mouse serum samples collected after Nipah-specific vaccination. We demonstrated that Nipah-specific infection/vaccination induces high-level neutralizing antibodies against homologous strains, with limited to no cross-reactivity neutralizing antibodies against antigenic distinct henipaviruses, thus highlighting the zoonotic potential of these closely related animal viruses. Convalescent serum samples collected from Nipah patients possess robust neutralizing antibodies against the Nipah virus but could not neutralize other ephrin-using henipavirus. Furthermore, mice vaccinated against Nipah-specific vaccines induce potent neutralizing antibodies against the Nipah Virus and Hendra Virus but not the Cedar Virus and Ghana Virus.

Poster 2

Intranasal dendritic cell-targeting vaccine booster elicits cross-clade, durable and protective systemic and mucosal immunity against sarbecoviruses

Nicholas You Zhi Cheang[1,2], Wee Chee Yap[1], Kirsteen McInnes Tullett[3], Xinlei Qian[2,4], Peck Szee Tan[3], Kiren Purushotorma[2,4], Wan Yi Tan[1], Shirley Yun Yan Mah[1], Paul Anthony Macary[2,4], Chee Wah Tan[1], Mireille Hanna Lahoud[3], Sylvie Alonso[1,2,5]

1 Infectious Diseases Translational Research Programme; Department of Microbiology & Immunology; Yong Loo Lin School of Medicine, National University of Singapore, Singapore
2 Immunology Programme, Life Sciences Institute, National University of Singapore, Singapore
3 Monash Biomedicine Discovery Institute & Department of Biochemistry and Molecular Biology; Monash University, Australia
4 Immunology Translational Research Programme; Department of Microbiology & Immunology; Yong Loo Lin School of Medicine, National University of Singapore, Singapore
5 National Centre for Infectious Diseases, Singapore

Short-lived, clade-specific immune responses with inadequate mucosal priming are limitations faced by current COVID-19 mRNA vaccines against sarbecoviruses. We developed a conventional type 1 dendritic cell-targeting nasal booster vaccine candidate (Clec9AOMNI) which consists of two recombinant Clec9A-specific monoclonal antibodies fused to the Receptor Binding Domain (RBD) from Omicron XBB.1.5 and SARS-CoV-1, respectively. We evaluated its immunogenicity and protective efficacy in mice that received prior Pfizer-BioNTech Comirnaty mRNA vaccination, compared to boosting with Omicron BA.4/5 bivalent Comirnaty (BC). Clec9AOMNI-boosted mice elicited broadly neutralizing antibodies (nAb) and T cell responses against both clade 1a and 1b sarbecoviruses, coupled with triple cross-reactive RBD-specific B cells that bind to ancestral SARS-CoV-2, Omicron XBB.1.5 and SARS-CoV-1 RBD. In contrast, the immunological breadth of BC-boosted mice was restricted to clade 1b. Excitingly, intranasal delivery of Clec9AOMNI generated robust mucosal immune responses in the upper and lower respiratory compartments, including IgA, cross-clade nAb, and cellular immunity accompanied by functional tissue-resident memory T cells. Furthermore, systemic and mucosal immune responses induced by Clec9AOMNI were highly durable and associated with the persistence of RBD-specific follicular T-helper cells, germinal centers, and increased long-lived plasma cell differentiation in the spleen and lungs. Correspondingly, Clec9AOMNI conferred sustained protection against Omicron BA.1 when challenge was performed at one- and six months post-boost. On the contrary, immunogenicity and protective efficacy waned in BC-boosted mice. Collectively, Clec9AOMNI triggered cross-clade, durable and protective systemic and mucosal immunity, supporting its translational application as a pan-sarbecovirus nasal booster vaccine that could potentially mitigate pandemic threats from emerging sarbecoviruses.

Poster 3

A dendritic cell-targeting approach to deliver a universal influenza vaccine candidate to the respiratory mucosa

Lee Zhang Wei Daryl[1,2], Kirsteen Tullett[3], Cheang Youzhi Nicolas[1,2], Mireille Lahoud[3], Sylvie Alonso[1,2]

1 Infectious Diseases Translational Research Programme,; Department of Microbiology & Immunology; Yong Loo Lin School of Medicine, National University of Singapore, Singapore
2 Immunology programme, Life Sciences Institute, National University of Singapore, Singapore
3 Monash Biomedicine Discovery Institute, Monash University, Australia

Seasonal influenza vaccines require annual updates due to antigenic drift and waning immunity, highlighting the need for a universal flu vaccine targeting conserved viral epitopes. The extracellular domain of M2 (M2e) is a leading universal vaccine antigen candidate, but its weak immunogenicity hinders clinical development. To enhance M2e immunogenicity, we developed a dendritic cell (DC)-targeting vaccine by fusing M2e to the heavy chains of a Clec9A-specific monoclonal antibody (Clec9A-M2e). We show that a prime-boost regimen with only 2µg of the Clec9A-M2e construct (adjuvanted with poly I:C) induced very high systemic M2e-specific IgG titres that afforded full protection against lethal H1N1/PR8 challenge in young adult mice, with minimal body weight loss up to 6 months post-boost. We also employed an antibody-dependent cell cytotoxicity (ADCC) reporter assay to demonstrate the functionality of immune sera collected. The Clec9A-M2e prime-boost immunization also generated significant M2e-specific T cell responses both systemically (spleen) and locally (lungs). In aged mice, Clec9A-M2e induced significant but lower M2e-specific antibody, T cell responses, and ADCC activity, correlating with only 50% protection and moderate lung viral reduction upon challenge. In young mice with pre-existing flu immunity, a single Clec9A-M2e dose boosted M2e-specific antibody titres to levels comparable to prime-boosted flu-naïve mice. Aged mice with pre-existing immunity required two doses of Clec9A-M2e to achieve similar immune responses. Taken together, these results support that the Clec9A-targeting strategy represents a promising vaccine delivery platform able to overcome the weak immunogenicity of M2e and induce strong immune responses upon respiratory immunization.

Poster 4

MARVAS110 blocks ULK1/2-mediated autophagy to inhibit Enterovirus D68 replication *in vitro* and *in vivo*

Yuhui Deborah Fong[1,2,3], Thinesshwary Yogarajah[2,3], Jasmaadiyah Binte Habib Mohameed[2,3], Justin Jang Hann Chu[1,2,3,4,5]

1 Integrative Sciences and Engineering Programme (ISEP), NUS Graduate School (NUSGS), National University of Singapore
2 Laboratory of Molecular RNA Virology and Antiviral Strategies, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore
3 Infectious Diseases Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore
4 Collaborative and Translation Unit for HFMD, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore
5 NUSMed Biosafety Level 3 Core Facility, Yong Loo Lin School of Medicine, National University of Singapore

Enteroviruses are a genus of small RNA viruses that cause diseases in both animals and humans, one of which being Enterovirus D68 (EV-D68). Unlike many other human enteroviruses, EV-D68 infection results in respiratory disease, which could develop into a serious condition termed acute flaccid myelitis, a complication leading to weakness in the muscles and reflexes. To date, there are no approved antivirals for the treatment of EV-D68 infection, highlighting the need to expand the search for antivirals. A phenotypic-based assay was developed to screen for kinase inhibitor compounds restricting EV-D68 infection. This led to the discovery of MARVAS110, which has potent antiviral activity against EV-D68 infection in the post-entry stages (IC50: 1.804 µM). To further elucidate the mechanism of action of MARVAS110, serial passaging of EV-D68, and siRNA knockdown studies were conducted, with results showing a possible link to the autophagy pathway via ULK1/2 inhibition. MARVAS110 reduces autophagosome formation in virus-infected cells, thereby preventing EV-D68 from hijacking the autophagy pathway to promote its replication. Furthermore, in an inaugural pre-clinical study, MARVAS110 treatment exhibited no observable toxicity and good efficacy. This novel compound holds significant therapeutic potential, offering a promising strategy for antiviral intervention and paving the way for the development of targeted treatments against a broad spectrum of enteroviral infections.

Poster 5

Role of mucosal associated invariant T Cells in the airway immune responses of an influenza infection

Zhe Zhang Ryan Lew[1], Hui Yi Tay[2,3], Jing Liu[4], Ser Mei Koh[5], Wendy Lee[5], Hyung Won Choi[2,3], Olaf Rötzschke[5], De Yun Wang[4], Justin Jang Hann Chu[1,6,7], Kai Sen Tan[1,6]

1 Infectious Diseases Translational Research Programme and Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore
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Seasonal strains of influenza impose continuous socioeconomic costs on society and zoonotic strains of highly pathogenic avian influenza are a constant concern. Due to the propensity of influenza to mutate, the virus-focused solutions are often insufficient to prevent infection while host-focused solutions, such as innate immune modulation, harbour untapped potential. Mucosal associated invariant T (MAIT) cells are activated in response to viral infections, accumulate at the infection site, and their activation in an influenza infection is associated with survival in in vivo mice models. These results suggest that MAIT cells may be harnessed to modulate the pathogenesis of influenza. However, as the activation of MAIT cells in SARS-CoV-2 patients is correlated with morbidity, they may contribute to immunopathology in some circumstances. Characterization of their functional characteristics may allow clinicians to harness MAIT cells therapeutically, while mitigating any immunopathology. PBMCs isolated from fresh blood was co-cultured with infected respiratory epithelial in an air-liquid interface culture. MAIT cells were then isolated from the PBMCs for single-cell RNA sequencing, flow cytometry evaluation, chemotactic propensity and programmed cell death. Our results indicate that human circulating MAIT (cMAIT) cells home towards and are activated in response to infected respiratory epithelial. Single-cell RNA sequencing also revealed pyroptosis as a potential pathway for immunopathology. Increased interleukin-1β and cleaved caspase-1 were also confirmed in lysates of cMAIT cells exposed to infected respiratory epithelial. cMAIT cells thus may undergo pyroptosis after exhibiting effector functions at sites of respiratory infection, an excessive amount of which may result in immunopathology.

Poster 6

Genome-wide siRNA screen for mosquito cellular factors involved in the replication of mosquito-borne viruses

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Mosquito-borne viruses are viruses capable of amplifying in mosquito vectors and spreading to humans through mosquito bites, resulting in large outbreaks. Examples of such viruses include ZIKV, CHIKV and DENV. While these viruses seldom lead to fatality, they result in debilitative consequences that reduce one's quality of life. Current treatment is limited to symptomatic relief. Hence, vector control strategies are essential to control potential outbreaks. An example is the production and use of genetically modified mosquitoes to reduce the wild vector population (population suppression) or the modification of the vector's competency to transmit these viruses (population replacement). With population suppression strategies, they can cause local extinction and risk reinvasion by neighbouring populations or species to occupy the same ecological niche. While such risk is absent from population replacement strategies, poor understanding of mosquito genes involved in the replication of these viruses makes it difficult to implement such strategies. Our study aims to discover a comprehensive list of mosquito cellular factors involved in the replication of these viruses through mosquito genome-wide siRNA screens. We also attempted to elucidate some of their mechanism of action. We hope that factors identified in this study would facilitate the formulation of new control strategies against these viruses.

Poster 7

Dynamic of CHIKV vgRNA in early infection

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Chikungunya virus (CHIKV), an arthropod-borne positive-sense RNA virus, continues to pose a global public health threat, with outbreaks reported in regions including Europe, the United States, and Singapore. Despite its growing impact, effective antiviral treatments remain limited. This study investigates the role of CHIKV viral genomic RNA (vgRNA) during the early stages of infection, with a focus on its interactions with host cell factors and the implications for viral replication. Using a multidisciplinary approach combining subcellular fractionation, confocal microscopy, transmission electron microscopy (TEM), and RNA fluorescent in situ hybridisation (FISH), the study examines how vgRNA is trafficked and stabilised within infected cells. A significant enrichment of vgRNA was observed in specific subcellular compartments early post-infection. These findings prompted further investigation into the role of the CHIKV capsid protein in regulating vgRNA dynamics. Mechanistic studies identified the capsid's nuclear localisation signal (NLS) as a key determinant of its intracellular trafficking behaviour. Inhibition of the host importin-α pathway using ivermectin significantly reduced vgRNA accumulation and impaired viral replication, suggesting a functional dependence on host trafficking machinery. In parallel, site-directed mutagenesis targeting the capsid NLS confirmed its importance in supporting efficient viral replication. High-resolution TEM further revealed close spatial association between vgRNA and capsid protein within infected cells. Finally, the study identified TDP-43, a multifunctional RNA-binding protein from the hnRNP family, as a vgRNA-interacting partner, suggesting a broader role for host RNA-binding proteins in CHIKV replication.

Poster 8

Key amino acids in structural envelope E protein drive the *in vivo* fitness and virulence of DENV2 cosmopolitan strains via immune evasion

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The pathogenesis of dengue virus (DENV) has remained elusive, hindering the development of antiviral drugs and effective vaccines. Previously, our lab showed that precursor-membrane and envelope protein (prME) play a critical role in driving the in vivo fitness of D2Y98P, a representative of DENV2 Cosmopolitan strains that circulate in Southeast Asia and cause recurrent outbreaks. This study aims to further characterise the role of prME in D2Y98P virulence and pathogenesis. We adopted a chimerisation approach that replaces prME from D2Y98P with that from non-virulent DENV2 NGC strain (NGC-D2Y chimera). The NGC-D2Y chimeric virus was strongly attenuated in IFNAR-/- mice, confirming the role of prME in driving the in vivo fitness of D2Y98P. Seven non-conserved amino acid substitutions were then introduced in NGC-D2Y to partially revert NGC prME sequence to D2Y98P sequence (NGC7-D2Y). Partial restoration of virulence was observed in IFNAR-/- mice with NGC7-D2Y displaying a bi-phasic viremia pattern. We further identified one additional amino acid (AA) substitution on E protein that could fully restore in vivo virulence of NGC7-D2Y. Importantly, the attenuated phenotype of NGC7-D2Y was totally abrogated in IFNAR-muMT mice, which lack B cells. This work demonstrated that the amino acid make-up in E protein drives the fitness and virulence of DENV2 Cosmopolitan strains by evading antibody-mediated viral clearance.

Poster 9

N153-linked glycans on envelope protein protect dengue virus from antibody-mediated clearance

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Dengue virus (DENV) poses a huge disease burden globally with an estimated 390 million infections annually. The envelope (E) protein of DENV is glycosylated at two highly conserved asparagine (N) sites (N67 and N153). The role and importance of these N-linked glycans in DENV pathogenesis has been elusive. A DENV2 mutant lacking N153-linked glycans (N153Q mutant) was engineered and found to be mildly impaired in vitro but drastically attenuated in a symptomatic mouse model of severe dengue, as evidenced by accelerated viral clearance. B cell depletion and knockout (KO) in mouse model restored N153Q parental virulence, suggesting the involvement of B cells in N153Q attenuation. Homologous passive transfer of purified IgM from infected B-cell proficient mice into B cell-KO mice cleared N153Q mutant from blood circulation. In vitro neutralization assay using mouse sera showed that WT and N153Q viruses were not significantly neutralized. These data suggest that N153Q mutant attenuation was due to IgM-mediated viral clearance involving a non-neutralizing mechanism. Interestingly, heterologous passive transfer of purified IgM from WT-infected mice into N153Q-infected B cell-KO mice did not clear the mutant, suggesting that infection with WT DENV and N153Q mutant induced distinct antibody repertoires. Together, our work has provided further insights into the role of N153-linked glycans on E protein in protecting DENV from a non-neutralizing IgM-mediated viral clearance mechanism. The findings represent a novel immune evasion strategy for DENV and have important implications for the development of antibodies, live attenuated vaccine and antiviral.

Poster 10

Exploiting the Clec9A targeting system to deliver a DENV subunit vaccine candidate

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With approximately 3.8 billion people at risk of infection in tropical and sub-tropical regions, dengue ranks among the top ten threats worldwide. Dengue places a large economic burden on endemic countries and has the potential for severe disease manifestation. However, dengue vaccine development has proven to be a challenge. Our group utilises the Clec9A-targeting system to develop a subunit DENV vaccine, which involves anti-mouse Clec9A antibody monoclonal antibody with a DENV2 envelope domain III (EDIII) genetically fused to the C-terminus of the heavy chain. Clec9A is a receptor that is specifically expressed in dendritic cells of the cDC1 subtype. cDC1s are highly efficient in processing antigens and cross-presentation on MHC I and MHC II, making Clec9A a promising DC surface receptor for antigen delivery. In addition, antibodies generated against EDIII are strongly neutralising and predominantly serotype-specific, minimising the risk of ADE.

Poster 11

Antiviral development for human enteroviruses

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Human enterovirus A71 (EV-A71) infection causes hand, foot, and mouth disease (HFMD) among young children, and is associated with severe neurological complications. It has emerged as a significant health concern due to the increasing frequency and severity of outbreaks globally. However, there is a lack of an internationally approved antiviral to manage EV-A71 infections. My study aims to fill this gap by identifying novel EV-A71 inhibitors using a high-throughput antiviral screening platform. We have identified 114 potential EV-A71 inhibitors from a screen of 11,475 bioactive compounds. Among these hits, MARVASBW001 was selected for downstream mechanistic studies based on its safety, efficacy, and novelty. The time course studies showed that MARVASBW001 acted on the post-entry stages of EV-A71. The results from NanoLuc assays using EV-A71 replication-competent and replication-defective replicons suggested that MARVASBW001 targeted viral RNA replication and protein translation. In vivo study demonstrated that MARVASBW001 achieved 100% protective therapeutic effect in EV-A71-infected murine model. The successful generation of drug-resistant mutants against MARVASBW001 revealed that it may directly target EV-A71. Furthermore, MARVASBW001 exhibited broad-spectrum antiviral activity against other species of enteroviruses (CV-A16, Echo7, CV-A24, and EV-D68). Altogether, these results suggested that the MARVASBW001 could be a potential antiviral candidate against EV-A71 infection.

Poster 12

Identification of prognosis and pathogenesis biomarkers in Enterovirus D68 infection

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Enterovirus D68 (EV-D68) has garnered attention over the recent past years with global outbreaks of acute respiratory infection in America and East Asia. EV-D68 can cause hospitalization and fatality due to asthma exacerbation and acute flaccid myelitis. However, the pathogenesis of EV-D68 is yet unknown and anti-viral drugs or vaccines are unavailable. Research on pathogenesis of EV-D68 infection is much needed. Here, we first successfully developed an in vitro air-liquid interface (ALI) culture model using human primary diseased bronchial epithelium cells- asthma (DHBE-As). Following that EV-D68 infection on ALI culture showed increase in 2-log units PFU/mL affirming the susceptibility of these cells to EV-D68 infection. With the aim to identify potential disease biomarkers and antiviral targets, we deployed the single-cell RNA transcriptomics sequencing approach on EV-D68-infected DHBE-As ALI culture. A total of 99 up-regulated genes and 123 down-regulated genes were identified. These genes were mainly involved in defense response to viruses, highly modulated genes throughout the asthma pathology, and antiviral innate immune responses. Hence, we chose to focus our aim on dissecting genes involved in host-virus interaction for further validation. Overall, we have successfully developed an ALI in vitro culture model for further dissecting the pathogenesis of EV-D68 and identification of potential prognosis biomarkers.

Poster 13

Development of sandwich ELISA using broadly reactive anti-nucleocapsid monoclonal antibodies for the detection of bat-borne merbecoviruses

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The zoonotic transmission of bat-borne betacoronaviruses, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2, has led to multiple severe human disease outbreaks. These events highlight the urgent need for enhanced surveillance tools capable of detecting betacoronaviruses with spillover potential. In this study, we generated three monoclonal antibodies (mAbs) targeting the nucleocapsid (N) protein using recombinant N proteins from SARS-CoV-2 and MERS-CoV. The cross-reactivity of these mAbs was assessed against a panel of representative betacoronaviruses. Based on their reactivity profiles, sandwich ELISAs (sELISAs) were developed to detect bat-borne betacoronaviruses. Among the mAbs, 7A7 exhibited the broadest cross-reactivity, recognizing viruses from the Sarbecovirus, Merbecovirus and Hibecovirus subgenera. An initial sELISA employing mAbs 7A7 and 6G10 successfully detected N protein in 75% (12/16) of clinical swabs from COVID-19 patients with Ct values < 25. To allow detection of bat-borne merbecoviruses, a second sELISA was developed using mAb 7A7 with mAb 8E2, which targets an epitope highly conserved among merbecoviruses. This assay demonstrated high sensitivity (limit of detection: 0.74 ng/ml) for recombinant N proteins from both MERS-CoV and the bat-borne HKU5-CoV. Notably, the epitopes recognized by 7A7 and 8E2 are also conserved in the recently identified HKU5-CoV-2, which utilizes human angiotensin-converting enzyme 2 (ACE2) for entry. These broadly reactive mAbs and their corresponding sELISAs lay the groundwork for the development of rapid antigen detection kits aimed at early detection of potential zoonotic spillover events, particularly in high-risk populations with close bat exposure.

Poster 14

Discovery of MARVASJH001 compound against respiratory viruses

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Respiratory viruses represent a considerable group of viruses that cause acute respiratory infections. These infections significantly impact morbidity and mortality worldwide, resulting in symptoms that range from a mild cold to severe pneumonia. The limited availability of effective antiviral treatments and vaccines emphasises the need for alternative strategies, such as drug repurposing. Drug repurposing is a promising approach as it utilises existing clinical data, thereby eliminating the lengthy development process from bench to bedside. To enhance its potency and efficacy, analogues of these drugs were synthesised, and a phenotypic assay was established, leading to the discovery of MARVASJH001. MARVASJH001 exhibited minimal cellular cytotoxicity >200 nM in both A549-hACE2 and A549 cell lines, compared to the control compound, MARVASJHC, which showed cytotoxicity > 10 nM and > 80 nM, respectively. Upon antiviral efficacy testing at 10 nM, MARVASJH001 demonstrated potent inhibition of 2.5 log-unit PFU/mL in SARS-CoV-2 in infected-treated A549-hACE2 cells compared to the control compound at 10 nM of MARVASJHC following 48 hours post-infection (hpi). In Influenza virus-infected A549 cells, 10 nM of MARVASJH001 showed 0.6 log-unit PFU/mL inhibition, similarly observed in the control compound, MARVASJHC, at 80 nm. The half-maximal inhibitory concentration (IC50) was determined to be 0.2320 nM for SARS-CoV-2 in A549-hACE2 cells and 0.1621 nM for the influenza virus in A549 cells, indicating high potency. Preliminary results suggest that MARVASJH001 increases viral inhibition in a dose-dependent manner, highlighting its potential as a novel treatment for respiratory viruses.

Poster 15

Development of a next-generation live-attenuated DENV2 vaccine strain using genome recoding

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Dengue virus (DENV) remains a major global health threat, causing millions of infections annually with no widely effective treatment. Live-attenuated vaccines are the most promising approach but face challenges due to complex serotype interactions and the risk of antibody-dependent enhancement (ADE). This research focuses on designing and validating a live-attenuated DENV2 vaccine strain by integrating two complementary strategies. First, genome recoding introduces numerous silent mutations to disrupt functional RNA structures and long-range interactions, reducing viral replication efficiency and generating an attenuated vaccine backbone. Second, non-silent, broad-spectrum attenuating point mutations—repurposed from successful orthoflavivirus vaccines—are strategically integrated into the recoded backbone. These mutations further attenuate the virus, while also enhance its immunogenicity, with the goal of stimulating robust adaptive immune responses. The candidate vaccine strains safety and efficacy were evaluated through rigorous in-vitro and in-vivo studies using suitable infection models. In vitro, compared to wildtype virus, the recoded strain exhibited reduced plaque size and replication kinetics in mammalian and mosquito cell lines. Furthermore, after screening, specific immunogenic point mutations which enhanced virus attenuation and increased innate immune response in the form of interferon production, were identified. In vivo studies in suckling mice confirmed attenuation, while experiments in AG129 mice demonstrated strong humoral immune responses, with vaccine-induced neutralizing antibodies protecting against lethal wild-type DENV2 challenge. This work contributes to DENV vaccine development by offering a novel, dual-strategy approach that enhances safety and immunogenicity. The findings support future tetravalent vaccine design, addressing limitations of current candidates and advancing global dengue prevention efforts.

Poster 16

The combination of Remdesivir and Ivermectin exerts highly potent and synergistic antiviral activity against murine coronavirus and SARS-CoV-2 infections

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The COVID-19 pandemic revealed deficiencies in our preparation against pandemic viruses, and highlighted the urgent need to develop more effective and broad-spectrum antiviral therapies against coronaviruses. One strategy to address this pressing concern is combination therapy using repurposed drugs with broad-spectrum antiviral activity against zoonotic viruses with pandemic potential. We previously demonstrated that the combination of remdesivir and ivermectin is highly potent and synergistic in inhibiting the replication of murine hepatitis virus (MHV), a betacoronavirus that represents a surrogate model for SARS-CoV-2. Time-of-addition and time-of-removal assays were performed to determine the viral replication processes likely affected by this drug combination. The interactions between this drug combination, virus and host processes were investigated by SWATH and bulk RNA sequencing of MHV-infected H2.35 mouse liver epithelial cells. The combination of remdesivir and ivermectin (at their respective IC50 concentrations) drastically diminished the coronavirus titer by 4 to 5 log10 greater than the respective monotherapies. Based on the proteomic and transcriptomic analyses, viral protein and RNA levels were significantly decreased upon combination treatment compared to the respective monotherapies. While remdesivir exhibited considerable negative effects upon host RNA processes, ivermectin resulted in the upregulation of host protein processes. Molecular pathways affected by the combination treatment were markedly distinct from the monotherapies, and indicated that ivermectin enhances the activity of remdesivir by modulating critical host processes to synergistically exert its inhibitory effect on the coronavirus replication cycle.

Poster 17

Sterilizing and vertical transmission-protective immunity induced by a live attenuated Zika virus vaccine in mice

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Rising global temperature, urbanisation, and migration has fuelled the global distributions of medically important mosquito-borne viruses, notably from the Orthoflavivirus family. In our own backyard, annual dengue virus (DENV) and sporadic Zika virus (ZIKV) outbreaks pose significant healthcare burden among local population. While the most recent ZIKV epidemic occurred just over a decade ago, sporadic ZIKV infections can still be detected in Singapore. With possible severe disease manifestation resulting from Zika virus infection, including congenital and developmental deformities and neurological disorders, coupled with the cyclical nature of Orthoflavivirus outbreaks, and in the absence of antiviral therapies, vaccination remains an important link in disease prevention. Utilising a novel approach, we developed a live attenuated vaccine, VacZen, through an extensive genome recoding approach. In short, this approach replaces codons extensively within the virus genome with synonymous mutations, resulting in a highly attenuated virus through disruption of intragenomic RNA-RNA interactions while maintaining the native amino acid sequence. This is critical in retaining the epitopes for immune cell presentation and maturation, leading to a robust protective efficacy. Our findings demonstrated a highly attenuated phenotype in vitro, and confers 100% sterilising protection in the immunocompromised AG129 murine model. Notably, VacZen addresses a paramount issue, conferring protection against the vertical transmission of ZIKV. Importantly, protection was achieved with a single dose vaccination regimen under lethal challenge conditions. These promising results supports the broader application of this approach to other medically important Orthoflaviviruses. The success of VacZen may represent a new paradigm in live attenuated vaccine design.

Bacteriology

Poster 18

Cross-complementation of the capsule polymerase in *Streptococcus pneumoniae* reveals insights into substrate-specificity, enzyme-function and serotype generation

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The Wzx/Wzy dependent pathway is a conceptually conserved synthesis pathway for cell-surface polysaccharides which exists across Gram-positive and negative bacteria species. The pathway can be used to describe the synthesis of O-antigen, enterobacterial common antigen, spore coat, teichoic acid and capsule polysaccharide (CPS) synthesis. A key component in this pathway is the Wzy polymerase. Wzy enzymes catalyze the polymerization of oligosaccharide repeat units. Very little information is known about Wzy activity, and it is widely held that these enzymes possess strict specificity. In S. pneumoniae, the CPS Wzy polymerase - referred to as CpsH, is conditionally essential. This fact, coupled with the genetic tractability of the bacteria, means that in S. pneumoniae the substrate specificity of these enzymes can be easily assessed. Here, we utilized a high-throughput approach to systematically examine the substrate specificity of 21 CpsH variants. We are able to show that these enzymes are not universally strictly specific, and that some variants are capable of polymerizing a range of oligosaccharide repeat units. A directed evolution experiment was performed, and we identified residues that are functionally important for enzyme specificity. Further, we demonstrate that the acquisition of a non-cognate CpsH variant can generate new CPS serotypes, either by changing the polymerization configuration or by facilitating modifications to the CPS by glycosyltransferases (GTs). Last, we discover that the expression of certain CpsH variants are toxic, which may be the driving force to maintain enzyme specificity. Overall, our work provides insights into Wzy activity and its role in serotype evolution.

Poster 19

Regulation of adhesin cbpA by *Streptococcus pneumoniae* two-component system

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Streptococcus pneumoniae is a respiratory pathogen responsible for about 30% of infection-related deaths globally in 2019. It causes diseases such as sinusitis, otitis media, bacteremia, and meningitis. Colonization of the human upper respiratory tract is a prerequisite for invasive pneumococcal disease (IPD). Factors influencing bacterial adherence to host epithelial cells include capsular polysaccharides (CPS) and adhesins such as choline-binding protein A (CbpA). Using A549 lung epithelial cells as a model, we confirmed that CPS reduced bacterial attachment. However, the extent of reduction correlated more closely with colony size than with CPS structure. To identify additional factors affecting binding, we employed random barcode transposon-site sequencing (RB-TnSeq). The screen validated several known adhesins, including CbpA, neuraminidase A (NanA), and pneumococcal adherence and virulence factor A (PavA). In addition, we found that transposon insertions in the response regulator vncR enhanced binding to A549 cells. RNA sequencing and genetic analyses uncovered unexpected crosstalk between the sensor histidine kinase VncS and RR06. This interaction, along with the upregulation of fructose-1,6-bisphosphate aldolase (Fba), led to increased CbpA levels and enhanced epithelial cell attachment. These findings provide new insights into the regulatory mechanisms and signaling pathways that govern respiratory tract colonization of S. pneumoniae.

Poster 20

High-throughput sequencing reveals interchangeability of capsule transporters in *Streptococcus pneumoniae*

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MOP (Multidrug/Oligosaccharidyl-lipid/Polysaccharide) family transporters are found in almost all life forms. They are responsible for translocating lipid-linked precursors across cell membranes to support glycan synthesis. While significant progress has been made in elucidating the transport mechanisms of MOP transporters, how they select their substrates remains elusive. Here, we explore the substrate specificity of the MOP transporters involved in the capsular polysaccharide (CPS) synthesis pathway of Streptococcus pneumoniae. Pneumococcal capsule flippases are highly diverse and transport over a hundred unique types of CPS cargo. Yet sequence similarity cannot predict whether they can functionally substitute for one another. We developed a high-throughput approach to systematically examine the transport of nearly 6,500 combinations of flippases and substrates. This was achieved through molecular barcode sequencing (Bar-seq) and saturated mutagenesis of a library of CPS flippase variants. To further expand substrate specificity, we employed fragmentase to generate recombinant flippase variants from single mutants previously identified with altered substrate recognition. This strategy successfully isolated gain-of-function variants capable of substituting for the essential peptidoglycan flippase YtgP (MurJ). Our study revealed novel pairs of non-cognate flippases and cargo, identified residues important for MOP flippase specificity, and demonstrated that specificity can be broadened through targeted mutations. These insights advance our understanding of MOP transporter selectivity and offer a promising tool for glycoengineering applications.

Poster 21

Ligation of capsular polysaccharides to peptidoglycan in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is an important respiratory pathogen that causes significant human invasive diseases. The key virulence factor of S. pneumoniae is the capsular polysaccharide (CPS), encasing the cell and protecting it from host immune responses. Majority of CPS biosynthesis of S. pneumoniae serotypes are mainly relied on Wzy/Wzx-dependent pathway. Although most of the enzymes responsible for the CPS synthesis were identified, the enzyme responsible for catalyzing this final ligation process is still uncertain. The potential ligase candidate is cpsA, the first gene encoded in CPS operon and a well-conserved protein found in almost all S. pneumoniae serotypes. CpsA belongs to the LCP (LytR-Cps2A-Psr) homologous enzyme family, in which lytR has been implicated in attaching wall teichoic acids (WTA) to PG in Gram-positive bacteria. The crystal structure and biochemical analysis of cps2A also demonstrated it carrying polyprenol pyrophosphatase activity. Deletion of LCP genes in different combinations in S. aureus and S. pneumoniae resulted in impaired growth and cell morphology and loss of large amount of CPS and WTA, further illustrating potential ligation function of LCP enzymes. Thus, we hypothesize that cpsA is the ligase for CPS attachment to PG and lytR may compensate for the function of cpsA function. We employ genetic and biochemical approaches to investigate the enzymatic activities of cps2A and lytR. Understanding ligase activity of LCP proteins is crucial for deciphering CPS synthesis of S. pneumoniae. By probing deeper into the molecular mechanism of these enzymatic activities, we could identify potential novel therapeutic or vaccine targets.

Poster 22

Genome-wide identification of essential gene suppressors in *Streptococcus pneumoniae*

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Identifying novel genetic interactions is paramount to uncovering unknown gene functions and elucidating connections between biological pathways. One interaction of interest is essential gene suppression, wherein an otherwise essential gene is rendered dispensable by a mutation in a separate 'suppressor' gene. Essential genes and their suppressors are often functionally linked, such that the function of the essential gene can suggest that of the suppressor gene, or vice versa. Here, we present suppressor transposon sequencing (Sup-TnSeq), an efficient and scalable technique to identify overexpression and loss-of-function suppressors of essential genes on a genome-wide scale. A query essential gene is deleted at saturation in a randomly barcoded transposon library to select for mutants with insertions suppressing their essentiality. Candidate insertions are subsequently identified via barcode sequencing (BarSeq). If the transposon design contains a strong outwards-facing promoter, recovered insertions displaying strand bias upstream of a gene indicate an overexpression suppressor, while those with no strand bias indicate knockout suppressors. We conducted a comprehensive Sup-TnSeq screen in Streptococcus pneumoniae by selecting for suppressors of seven essential genes. The screen recapitulated almost all known suppressors for the queried genes and revealed several novel candidate suppressors, which we have also independently validated. Additionally, we performed mechanistic studies on previously unknown suppressors of three essential genes: ackA (acetate kinase), yqeH (30S ribosomal biosynthesis protein), and cdsA (phosphatidate cytidylyltransferase).

Poster 23

Dual randomly barcoded transposon sequencing (Dual Tn-seq) profiles the genetic interaction landscape in bacteria

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Gene redundancy often complicates systematic approaches to characterizing gene functions because single gene deletions may not produce discernible phenotypes. Thus, despite the advent of next-generation sequencing, over 11 million microbial genes in the NCBI reference sequence database have no known function. In this study, we report Dual Tn-seq, a novel platform for comprehensively assaying the fitness of a large pool of double mutants in parallel. Dual Tn-seq couples random barcode transposon-site sequencing (RB Tn-seq) with the Cre-lox system, enabling deep sampling of ~1.4 billion double mutants in the human pathogen Streptococcus pneumoniae. This work captured 73% of the 1.3 million combinations of gene deletions that could theoretically be made in a single genome. The genetic interactions identified spanned a wide range of biochemical processes, revealing new factors in presumably well-studied pathways, exemplified by a novel CTP synthase PyrJ. Moreover, double-mutant libraries should permit investigation of a broader range of genetic interactions by growth in other conditions. Since Dual Tn-seq does not require the construction of a large array of single mutants, it should easily adapt to various microorganisms.

Poster 24

Regulatory role of PTP4A2 in antibacterial immunity

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PTP4A2 (Protein tyrosine phosphatase 4A2, PTP4A2) has been implicated in the antibacterial immune response. However, the role and underlying mechanism of PTP4A2 in antibacterial immunity remain unclear. PTP4A2 knock-out (KO) macrophages and overexpression (OE) macrophages were generated to investigate the role of PTP4A2 in immune response to Klebsiella pneumoniae infection. A CFU assay was used to measure the bacterial load in PTP4A2 KO/OE macrophages. In addition, the activation of MAPKs (ERK1/2, p38 and JNK), IRF3 and NF-κB at various time points were examined using western blot. The expression of proinflammatory cytokines and IFN-β was assessed through qPCR and ELISA. Bacterial load was found to be reduced in PTP4A2 OE macrophages, increased in PTP4A2 KO macrophages compared to their respective control macrophages. PTP4A2 KO resulted in decreased activation of p65, p38 and ERK, whereas PTP4A2 overexpression enhanced ERK activation. Examination of cytokine expression showed that PTP4A2 deficiency resulted in reduced expression of IL6, TNFα and IL1β, and overexpression led to increased expression of these cytokines compared to control macrophages in response to Klebsiella pneumoniae infection. In macrophages, PTP4A2 expression enhances MAPK and NF-κB signalling, and upregulates the expression of IL-6, TNFα and IL1β in response to Klebsiella pneumoniae infection. Additionally, PTP4A2 promotes the bactericidal activity of macrophages.

Poster 25

Development of a bioelectric device for disinfection of hospital sink biofilms

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Hospital sinks are reservoirs for biofilm-forming multidrug-resistant organisms (MDROs) which cause healthcare-associated infections (HAIs). Conventional mitigation methods, like chlorine disinfection and sink trap replacement, often fail due to persistent biofilm regrowth. We evaluated Bioshield Pro (BPro), a bioelectric disinfection prototype, through laboratory testing and a real-world paediatric clinic trial. In vitro testing assessed the effectiveness of BPro, alone and with 1000 ppm chlorine, against Enterococcus faecalis, a common and resilient HAI organism. A three-month field trial was also conducted. Biofilm formation in sink bottle traps was assessed using crystal violet staining. Bacterial splashback was quantified using colony counts from agar plates placed near the sink strainer, simulating hand contamination from water droplets. Laboratory tests showed that applying 1.0V reduced E. faecalis to 39.9% ($p \leq 0.001$) relative to the control. BPro also augmented chlorine disinfection, lowering bacteria presence from 63.9 % to 29.9 % ($p \leq 0.01$). In the clinical setting, BPro reduced biofilm to 12.1 % relative to the control ($p \leq 0.0001$), with no added benefit from combining with disinfectants. Additionally, BPro reduced bacterial splashback by over 80.0% (from 17.67 CFU/plate in the control sink to 2.67 CFU/plate), outperforming chlorine treatment alone (13.67 CFU/ per plate). The system operated continuously at low voltage, with no reported adverse effects during the trial. These preliminary tests demonstrate that BPro is a promising alternative to conventional healthcare sink disinfection. Further validation in diverse clinical environments is needed to support broader implementation. To facilitate clinical integration, modular and scalable system designs are under development.

Poster 26

YopJ inactivity unravels caspase-11 activation during Yptb infection

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To establish an infection, pathogenic microorganisms such as extracellular Yersinia pseudotuberculosis (Yptb) uses an acetyltransferase, YopJ, to inhibit TAK1 and subvert MAPK and NF-kB signalling pathway. To counteract bacterial blockade of TAK1 signalling, macrophages assemble a cytosolic multiprotein complex comprising RIPK1, FADD and caspase-8 to trigger a gasdermin-D (GSDMD) dependent form of lytic cell death known as pyroptosis. However, how macrophages respond to Yptb infection in the absence of YopJ or caspase-8 activity is currently unclear. Here, we demonstrated that the catalytic inactive YopJ (YopJC172A) actives cytosolic LPS sensor, caspase-11, to drive pyroptosis, with caspase-11 being seen as a requirement following in vivo infection. We further noted that Yptb triggers caspase-11 dependent cell death under conditions of caspase-8 deficiency, with Yptb invading into the cytosol of macrophages. Therefore, our results highlights the plasticity of cell death pathway to ensure GSDMD activation to drive an anti-Yptb defence.

Poster 27

A bacterial network of T3SS effectors counteracts host pro-inflammatory responses and cell death to promote infection

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Innate immune signalling and cell death pathways are two highly interconnected processes involving receptor-interacting protein kinases (RIPKs) to mediate potent anti-microbial responses. To subvert these host responses, pathogenic bacteria inject a wide range of virulence factors known as type 3 secretion system (T3SS) effectors. However, whether there is any interplay between bacterial T3SS effectors, and what are the underlying mechanisms for the host to retaliate these bacterial subversion mechanisms are not completely understood. Here, we demonstrate that upon Citrobacter rodentium infection, murine bone marrow-derived macrophages activate a pro-inflammatory form of caspase-8-mediated cell death, requiring RIPK1 kinase activity, to counteract NleE effector blockade of pro-inflammatory gene expression. While C. rodentium injects a second effector, NleB, to suppress RIPK1-caspase-8 signalling, macrophages elicit an alternative form of cell death that is RIPK3 kinase-dependent, known as necroptosis, which occurs only upon caspase-8 blockade or deficiency. To counteract the host, C. rodentium translocates a third effector, EspL, to cleave and inactivate RIPK1 and RIPK3 from activating necroptosis. Furthermore, we also elucidate that NleB and EspL show additive effects in suppressing caspase-8-driven apoptosis and NLRP3 inflammasome activation in macrophages when necroptosis fails to take place. Taken together, our findings demonstrate the evolutionary importance of cell death pathways for anti-bacterial defence, which may have driven bacterial pathogens to express a complex network of effectors for adapting to the host-pathogen arms race. Future work understanding the effector response of cell death will provide novel insights into infectious and inflammatory pathologies.

Parasitology

Poster 28

Perturbation of vagus-linked metabolites by a gastrointestinal parasite

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Blastocystis is a highly prevalent gut parasite whose pathogenicity remains unclear. Both beneficial and detrimental effects have been observed as a result of Blastocystis infection, including altered gut microbiota, metabolism, and gastrointestinal health. Our prior work shows that this parasite expresses a modified tryptophanase enzyme dubbed BhTnaA, which has the unique ability to metabolize indole to tryptophan. Enterochromaffin cells in the gut use tryptophan as a precursor in the enteric synthesis of serotonin, and are innervated by the vagus nerve, an essential mediator of signaling between the gut and the brain. The vagus nerve is susceptible to changes in gut serotonin. Perturbed serotonin signaling has been associated with disorders linked to gut-brain axis dysfunction, such as Irritable Bowel Syndrome and some mood disorders. Our study shows that Blastocystis can use BhTnaA to influence serotonin synthesis by enterochromaffin cells in vitro and in a mouse model, and that this results in alterations to mouse behaviour.

Poster 29

Unveiling *Blastocystis*: dual roles in gut inflammation and metabolic health

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Blastocystis, a common and diverse protist in the human gut microbiota, has emerged as a potential regulator of gut and metabolic health. Once considered merely a commensal or pathogen, recent studies reveal subtype-specific roles that can either promote or impair host health. Epidemiological evidence links Blastocystis colonization to increased microbial diversity, healthier dietary patterns, and improved cardiometabolic profiles. Mechanistically, it may influence host physiology by modulating gut microbiota composition, enhancing short-chain fatty acid (SCFA) production, and shaping immune responses. Certain subtypes appear to strengthen epithelial barrier integrity and induce anti-inflammatory effects, while others may contribute to gut inflammation, particularly in the context of inflammatory bowel disease (IBD). Additionally, Blastocystis may impact the gut-brain axis through its role in tryptophan metabolism and microbial signaling. Despite these promising associations, causal relationships remain unclear, highlighting the need for mechanistic studies using axenic cultures, in vitro co-culture systems, and animal models. This poster presents current insights into Blastocystis's dual roles in health and disease and proposes a research framework to unravel its complex biology. Understanding this protist's context-dependent behavior may unlock new opportunities for microbiome-based diagnostics and therapeutic interventions.

Poster 30

Mitochondrial calcium as the initiator of high dose chloroquine-induced cell death phenotype in *Plasmodium falciparum*: implications for novel antimalarial therapies

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Understanding the mechanism of action of novel antimalarial drugs is a key goal in eradicating malaria. Previously, we have explored an alternative cell death mechanism elicited by micromolar amounts of the antimalarial chloroquine (CQ) against Plasmodium falciparum. The phenotypes assessed were calcium redistribution, mitochondrial depolarization and parasite digestive vacuole membrane permeabilization. Identifying the source or initiator of calcium release in the parasite could reveal new therapeutic targets. Timepoint studies revealed simultaneous mitochondrial integrity loss and intracellular calcium dysregulation, followed by membrane rupture. Using a series of calcium efflux inhibitors and antioxidants, mitochondrial calcium (Ca2+mt) emerged as a trigger for these parasite cell death phenotypes. Having established the mechanism with a high dose concentration of CQ, we screened the Medicines for Malaria Venture (MMV) Pathogen Box for similar effects. Three “hit” compounds were further characterized, with MMV085071 being the top performer. Ten analogues based on the top hit were assessed with dose-response assays and MMV1545561 was selected as the top candidate based on its efficacy. Both the parent and lead compounds were investigated for their ability to redistribute Ca2+mt. Although statistically significant, the lack of complete reversal of compound-induced calcium dysregulation and mitochondrial collapse by inhibitors suggested that Ca2+mt’s role is more limited as compared to that of chloroquine. Additionally, the transcriptomic study indicated that the novel screening compounds likely target multiple parasite pathways. Given their high efficacy, further detailed investigations are required to fully elucidate their mechanisms of action.

Poster 31

Rewiring of protein trafficking and translocation drive Ivermectin tolerance in *Plasmodium falciparum*

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Ivermectin (IVM), a safe, mosquitocidal endectocide, has shown promise as a complementary agent in malaria combination therapies and has been shortlisted for development under MMV's TCP6 initiative. While IVM-sensitive Plasmodium falciparum parasites (3D7) exhibit developmental arrest and trophozoite-stage death, IVM-tolerant lines selected in vitro reveal significant morphological and transcriptomic adaptations that help maintain survival under drug pressure. Although IC_{50} values remain unchanged, tolerant parasites withstand sustained IVM exposure, indicating enhanced tolerance rather than resistance. Morphological profiling using scanning transmission electron microscopy (STEM) revealed that IVM disrupts protein trafficking, leading to dilation of the parasitophorous vacuole (PV), ribosome depletion, and alterations in knob structures essential for cytoadherence—likely impairing the surface display of adhesion ligands. Tolerant parasites, however, resolve PV dilation, maintain normal knob morphology, and sustain replication under constant IVM pressure, albeit with reduced fitness. STEM analysis further revealed altered organelle architecture including segregated digestive vacuoles, suggesting remodeling of intracellular trafficking. Genomic sequencing identified SNPs in Cytochrome b (G131S) and a Symplekin-domain protein (S1018L) linked to reduced susceptibility. Transcriptomic data showed downregulation of the exportome, including surface antigens, and alteration of transport at translocation complexes of SRP, Sec and potentially PSAC. These changes suggest that IVM is driven by the rewiring of intracellular trafficking and export patterns, with PSAC components as potential drug targets. These findings provide critical insights into IVM's antimalarial action, the parasite's adaptive response to sustained drug pressure, and inform the rational design of combination therapies to delay tolerance and prevent resistance emergence.

Poster 32

Chloroquine induces eryptosis in *Plasmodium falciparum*-infected red blood cells and the release of extracellular vesicles with a unique protein profile

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Malaria is a vector-borne parasitic disease that affects millions globally. To achieve the goal set by the World Health Organization of reducing malaria cases by 2030, antimalarial drugs with novel mechanisms of action are required. Chloroquine (CQ), a classic antimalarial, was previously shown to trigger features of programmed cell death in Plasmodium falciparum, mainly characterized by calcium efflux from digestive vacuole (DV) permeabilization. Elevated intracellular calcium induces suicidal death of erythrocytes, known as eryptosis. This study investigated the hallmarks of eryptosis caused by calcium redistribution and the downstream cellular effects of CQ treatment in infected red blood cells (iRBCs). Using P. falciparum 3D7 mid-late trophozoites, we observed increased phosphatidylserine (PS) exposure, cell shrinkage, and membrane blebbing, delineating an eryptotic phenotype in the host RBCs. Notably, the outward budding and blebbing of the iRBCs plasma membrane forms extracellular vesicles (EVs) which are complex structures with specialized functional properties. Proteomic analysis of EVs from CQ-treated iRBCs revealed highly enriched proteasome and ribosome protein clusters. Although these EVs did not affect parasite growth, they appeared to activate interferon (IFN) signaling pathways mediated by IL-6 in THP-1-derived macrophages. Our findings demonstrate that CQ induces eryptosis in iRBCs accompanied by the release of EVs with a unique cargo, providing new insights into parasite-host interactions and immune modulation specifically driven by drug treatment.

Poster 33

Role of single cell eukaryotes on the host gut microbiome: investigations on encapsulation properties of *Blastocystis* ST4 as a step towards understanding its probiotic properties

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Blastocystis is an anaerobic intestinal protistan parasite, belonging to the stramenopiles, that inhabits the human and animal intestine and leads to an infection called blastocystosis. It can trigger various gastrointestinal system issues such as, abdominal pain, bloating, diarrhoea, indigestion, nausea and vomiting. However, studies conducted have demonstrated the beneficial effects of Blastocystis ST4 on host gut health. This experiment aims to investigate the possibility of encapsulating Blastocystis ST4, the viability of encapsulated Blastocystis ST4; and the benefit of the consumption of the encapsulates on the host's gut health. Blastocystis ST4 undergoes encapsulation within calcium alginate spheres through sodium alginate and calcium chloride chemical interactions. To optimise viability, experiments using different solutions of Iscove's Modified Dulbecco's Medium (IMDM), Horse Serum and Sodium Thioglycolate were conducted. Encapsulates are stored in anaerobic jars at room temperature for up to 72 hours. The encapsulate are chelated using EDTA and Sodium Citrate Solution in an incubator at various timepoints. The cells released from the encapsulates are washed and stained with Propidium iodide (PI) for flow cytometry. Results: (1) Blastocystis ST4 can be encapsulated within Calcium Alginate combined with Sodium Thioglycolate. (2) Encapsulated Blastocystis ST4 has ≥ 55% viability in anaerobic jars at room temperature at 72H timepoint. For impending work using mouse models, we expect to see encapsulated Blastocystis ST4 prevents loss of microbiota diversity, increase the proportion of Clostridia vadinBB60 group and Lachnospiraceae NK4A136 group contributes to attenuation of experimental colitis similar to the gavage route.

Poster 34

Unraveling the molecular mechanisms of a gut parasite, *Blastocystis*

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Blastocystis, a common gut parasite, is traditionally associated with gastrointestinal disorders like Irritable Bowel Syndrome (IBS) and Colorectal Cancer (CRC). However, recent research highlights significant subtype-specific differences. Subtype 7 (ST7) worsens gut inflammation and disrupts the intestinal barrier, while Subtype 4 (ST4) unexpectedly protects gut health. Despite these findings, the molecular mechanisms underlying these effects remain unclear. This research identifies and characterizes ST4-specific proteins, focusing first on tryptophan metabolism, critical for mood regulation and gut antimicrobial activity. Structural comparisons revealed differences between ST4 and ST7 tryptophanases, suggesting that ST4 preserves serotonin synthesis and antimicrobial functions, that were previously found to be disrupted by ST7. Functional assays with recombinant tryptophanases are underway to confirm its beneficial role. We also identified a unique ST4 protein named "CPI," resembling cysteine protease inhibitors. Given cysteine proteases' role in damaging intestinal barriers, CPI may represent a therapeutic target. ST4 exhibits lower proteinase activity than ST7, thus, protease inhibition assays will further investigate CPI's protective role. Additionally, we study L-asparaginase, a laterally transferred bacterial protein known for immune evasion in pathogens like Helicobacter pylori. Structural analyses suggest that ST4's L-asparaginase variant may differ functionally. Using molecular cloning, protein expression, and enzymatic assays, this study seeks to clarify ST4's beneficial mechanisms, potentially revealing novel therapeutic targets for gastrointestinal and mood disorders.

Poster 35

Methylene blue treatment of fatal cerebral malaria and identification of potential blood biomarkers

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Cerebral malaria (CM) is a severe complication caused by Plasmodium falciparum infection, leading to persistent neurological impairments in survivors. To understand the complex mechanisms and investigate advanced diagnostic and treatment strategies targeting human CM, we utilized Plasmodium coatneyi-infected rhesus macaques, a non-human primate model closely resembling P. falciparum infection in humans. Through differential gene expression analysis, our study demonstrated methylene blue's efficacy (>10 mg/kg) in reversing the detrimental effects of infection on the brainstem. Furthermore, by comparing our brainstem dataset from P. coatneyi-infected Macaca mulatta with two additional transcriptomic datasets (P. coatneyi-infected M. mulatta blood and P. falciparum-infected human blood), we identified nine genes associated with CM severity. Most of these genes were expressed in neutrophils, indicating their potential as blood biomarkers for diagnosing P. falciparum-induced fatal CM. This research highlights the necessity for new CM treatments and reveals promising biomarkers that could improve diagnosis and prognosis in affected individuals.

Poster 36

Experimental reverse zoonosis of *Plasmodium falciparum* in mouse erythrocytes reveals key cellular pathways for anti-malarial drug discovery

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Malaria is a life-threatening disease that continues to pose a major global health challenge, impacting millions of individuals each year and is caused by parasites of the Plasmodium species. Of the five known human-infecting species, Plasmodium falciparum remains the most extensively studied due to its high prevalence and association with severe clinical outcomes. . P. falciparum is able to invade mouse red blood cells (mRBCs) but fails to complete its maturation cycle within this non-native erythrocytic environment. We aimed to gain insights into the species barrier between human and mouse erythrocytes in order to identify crucial cellular pathways for drug discovery by using mRBCs infected with P. falciparum. To achieve this, we conducted microarray hybridisation followed by comprehensive transcriptomic analyses, including both differential gene expression and weighted gene co-expression network analyses. The functions of the key differentially-expressed genes highlight pathways potentially responsible for impaired ring stage development observed in mRBCs, and serves as a stepping stone to develop new drugs against P. falciparum.

Immunology

Poster 37

Deficiency of DUSP4 potentiates colitis-associated colorectal cancer via CD8+ T cell dysfunction

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The coordination between immune responses and epithelial integrity is pivotal to the pathogenesis of inflammation-associated colorectal cancer (CRC). However, the molecular mechanisms that bridge immune cell dysfunction and epithelial transformation remain incompletely defined. In this study, we identify dual-specificity phosphatase 4 (DUSP4) as a key immune cell-intrinsic regulator of MAPK signaling that mitigates intestinal inflammation and suppresses tumorigenesis. Notably, analysis of clinical specimens reveals that high DUSP4 expression correlates with improved overall survival in CRC patients, suggesting a protective role of DUSP4 in CRC. Using CRC murine models, we demonstrate that deficiency of DUSP4 exacerbates colitis and accelerates the development of inflammation-driven CRC. Mechanistic investigations employing bone marrow chimeras reveal that the tumor-suppressive function of DUSP4 is intrinsic to the hematopoietic cells and is closely associated with robust cytotoxic T cell responses. Further functional analyses show that DUSP4 is essential for the activation, proliferation, and degranulation of CD8+ T cells, underscoring its role in maintaining effective anti-tumor immunity within the intestinal microenvironment. These findings suggest that DUSP4 is an important regulator of CD8+ T cells in anti-tumor immunity in the gut, highlighting the potential of this molecule as a prognostic biomarker and immunomodulatory target in inflammation-associated CRC.

Poster 38

DEFA5-producing CD4+ T cells in the intestines of atopic dermatitis patients play an important role in the development of AD-associated intestinal inflammation

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Atopic dermatitis (AD) is associated with various gastrointestinal symptoms, with underlying mechanisms remain poorly understood. In this study, through analysis of the cellular atlas of the intestines of patients with AD, we identified specific groups of CD4+ intraepithelial lymphocytes (IELs) capable of producing defensin alpha 5 (DEFA5) in the small intestine, and this groups of IELs may play important roles in mediating the development of intestinal inflammation in AD. Single-cell RNA sequencing was employed to analyzed immune cells composition in the ileum of AD patients. A pronounced upregulation of DEFA5 in CD4+ T cells in AD patients were identified. The DEFA5-expression CD4+ T cells were enriched in tissue central memory T cells (Tcm) and tissue resident memory T cells (Trm). It was also found that the peroxisome proliferator-activated receptor (PPAR) signaling pathway may play an important role in the expression of DEFA5 by CD4+ IELs. Using a mouse model of AD, it was confirmed that AD was associated with increased DEFA5-expressing CD4+ IELs which contributes to the damage of intestinal barrier function and the development of inflammation. These findings indicate that AD was associated with an increase in intestinal DEFA5-expressing CD4+ IELs which may play an important role in the development of intestinal inflammation.

Poster 39

Characterizing neutrophils and macrophages in an acute inflammation lung model in mice

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Neutrophils are first responder immune cells to infection sites. They are usually cleared by macrophages afterwards, in a process known as efferocytosis. Excessive neutrophil influx to and failed neutrophil clearance from infection sites would therefore cause further tissue injury. Resolution, an active process involving the production of specialised pro-resolving lipid mediators, has been found to induce efferocytosis and the recruitment of pro-resolving macrophages, allowing subsequent tissue repair. Resolution is therefore the key for recovery. However, there is limited documentation on exactly when efferocytosis occurs. This study seeks to determine when efferocytosis occurs after infection, and the effects of any potential sex differences on resolution responses. Mice would be treated with LPS to induce inflammation before being sacrificed at different time points across 10 days. Wet and dry lungs were weighed to assess the extent of oedema as inflammatory response, while fluids from lungs were analysed using flow cytometry to determine immune cell counts. The results showed a clear neutrophil influx that peaked 2 days after infection, coupled with the highest efferocytosis events observed. Inflammation generally resolved by day 8, but macrophages maintain at a high level while neutrophil levels returned to baseline. Efferocytosis peak could range from day 1 to 3, indicating efferocytosis as an early and fast response. Sharp decrease of neutrophil frequency at day 2 suggests the presence of several different neutrophil clearing mechanisms without efferocytosis being the main contributor. Future work can explore the molecular mechanisms that induce efferocytosis, and movements of macrophages and neutrophils during resolution.

Poster 40

Determining the role of RIPK3 in TLR-induced cell death

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Toll-like receptors (TLR) 3 and 4 sense viral double stranded DNA (dsDNA) and bacterial lipopolysaccharide (LPS) respectively. Activation of TLR3 and TLR4 initiates pro-inflammatory and pro-survival signalling through the adaptor, TIR-domain-containing adapter-inducing interferon-β (TRIF). This signalling pathway is tightly regulated by checkpoints such as cellular inhibitors of apoptosis proteins 1/2 (cIAP1/2) and IκB kinase α/β (IKKα/β). When these checkpoints are inhibited by drugs or microbial effectors, TRIF assembles a pro-death complex consisting of caspase-8 to trigger apoptosis. Emerging studies have revealed that receptor-interacting serine/threonine-protein kinase 3 (RIPK3), a kinase best characterised for driving necroptosis, a lytic and inflammatory form of programmed cell death, also promotes apoptosis when cIAP1/2 are inhibited in TLR4-stimulated cells. However, whether RIPK3 drives apoptosis under conditions of IKKα/β inhibition and cIAP1/2 depletion in TLR3-stimulated macrophages remains unclear. Here, we demonstrate that RIPK3 promotes apoptosis downstream of cIAP1/2 but not IKKα/β inhibition in both TLR3 and 4-stimulated macrophages, suggesting that the pro-death complexes formed are distinct following cIAP1/2 and IKKα/β inhibition. Thus, future work will determine the mechanisms behind the differential RIPK3 dependency and may uncover novel therapeutics for antimicrobial defence.

Notes

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