14TH ANNUAL SOUTHERN CALIFORNIA EUKARYOTIC PATHOGEN SYMPOSIUM

NOVEMBER 20, 2024
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Cover Image
Katherine Ralston, Ph.D.
University of California, Davis
Entamoeba histolytica performing trogocytosis (cell-nibbling) on a human cell.

Welcome to the 14th Annual Southern California Eukaryotic Pathogen Symposium

The study of eukaryotic pathogens includes such diverse organisms as intracellular protozoa, helminths, and fungal pathogens. As with previous years, the annual Southern Californian Eukaryotic Pathogen (SCEP) Symposium brings together researchers from over 20 different labs, representing four UC campuses, several Cal State and local universities, and many visitors from out of state, including SCEP alumni. All participants are united in investigating the intricate mechanisms and host responses associated with these significant pathogens. The aim of this symposium is to connect like-minded yet individual groups, fostering interaction, collaboration, and an appreciation for groundbreaking science.

This year's keynote speaker is **Dr. Sarah L. Gaffen**, the Gerald P. Rodnan Professor in the Division of Rheumatology & Clinical Immunology at the University of Pittsburgh. Dr. Gaffen will present "**All Things Great and Seventeen: At the Crossroads of IL-17 Signaling in Fungal Immunity and Beyond**," highlighting her lab's transformative work on IL-17 signaling and its critical role in immune responses to fungal pathogens and beyond. We are also excited to have one of our own SCEP veterans: **Dr. Kent Hill**, Professor of Microbiology, Immunology, and Molecular Genetics at the University of California, Los Angeles, who will deliver the morning invited talk titled "**Dynein Dragon Boat: Atomic Structure of the Trypanosome Axoneme Illuminates Mechanism of Flagellar Beating in Eukaryotes**." Dr. Hill's research provides cutting-edge insights into the molecular mechanisms that drive flagellar movement in eukaryotic cells, with implications for understanding diverse biological processes.

Congratulations to **Katherine Ralston** and **Wesley Huang** for winning the cover image competition with the striking image of "*Entamoeba histolytica* performing trogocytosis on a human cell." The image captures live imaging of an amoeba (cell tracker, turquoise) taking bites (arrowheads) from a human Jurkat cell (CD59-mCherry, orange). Live Z-stacks were collected on a Zeiss 980 with Airyscan2.

A heartfelt thank you goes to **Pica Preston** and her team for their invaluable coordination of this symposium, and to our dedicated student volunteers for ensuring the event runs seamlessly. We are also grateful to the Nair and Le Roch Lab (Greg Isales, Victor Chiang, Arrmund Neal, George Tseng, Loic Ciampossin) for all their help with the event.

To all past attendees and alumni who have moved on to incredible achievements, we deeply appreciate your contributions. You have helped make this symposium an inviting, productive, and successful event for everyone.

Karine Le Roch and Meera Nair
2024 SCEP Organizing Committee

Keynote Speaker: Sarah Gaffen, PhD., Gerald P. Rodnan Professor
President, International Cytokine & Interferon Society (ICIS)
Director of Basic Rheumatology Research
Division of Rheumatology & Clinical Immunology
University of Pittsburgh

All Things Great & Seventeen: At the Crossroads of IL-17 signaling in autoimmunity versus host defense

Biography

Dr. Sarah Gaffen is the Gerald P. Rodnan Endowed Professor in the Division of Rheumatology & Clinical Immunology, at the University of Pittsburgh. Dr. Gaffen did her undergraduate training at Carnegie Mellon University and received her PhD from The University of California, Berkeley under the guidance of National Academy of Science member Dr. Marian Koshland. Dr. Gaffen did postdoctoral work at UC San Francisco and was on the faculty at SUNY Buffalo from 1999-2008, where she initiated her work on defining mechanisms of signaling by the then-enigmatic IL-17 receptor cytokine family. Since 2008 she has been at the University of Pittsburgh, and was honored with the Gerald P. Rodnan Chair in 2015. Dr. Gaffen is one of the pioneers of studies of the signaling functions and structural features of the IL-17 receptor. The Gaffen Lab also works on understanding the basis for immunity to infections and autoimmunity, with a major interest in the mechanisms that underlie oral mucosal immunity and antifungal host defense. Her group was the first to demonstrate a role for IL-17 pathways in immunity to mucosal Candida albicans infections. Additionally, her recent work has uncovered important post-transcriptional pathways that determine IL-17 signaling cascades. Dr. Gaffen has published over 140 papers and Chaired the standing NIH study section “Immunity and Host Defense.” She was awarded the 2020 BioLegend/William E. Paul Award from the International Cytokine & Interferon Society and was elected a Fellow of the American Academy of Microbiology and the AAAS. Dr. Gaffen has been continually funded by NIH since 2001, and has mentored 13 students to completion of a PhD. Her trainees work all over the world in both academia and industry.

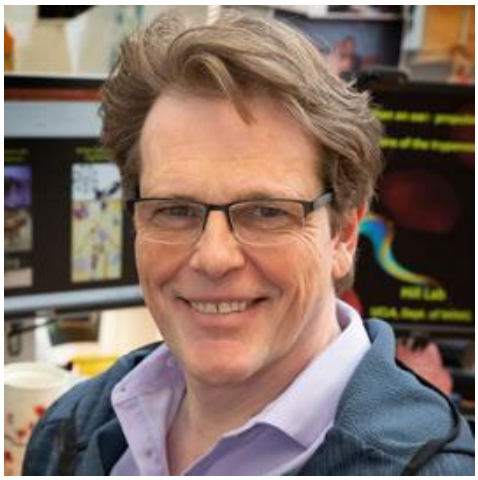
Research Interests:

T cell derived cytokines are critical for mediating host defense against infectious disease, but they also mediate disease pathology in autoimmunity. A subset of CD4+ T cells, known as "Th17 cells" based on production of the cytokine IL-17, plays a key role in driving autoimmunity. Conversely, IL-17 and Th17 cells play important roles in fungal immunity, particularly in protection against opportunistic mucosal infections caused by the commensal yeast Candida albicans, first shown by Dr. Gaffen's group. IL-17 and its receptor are unique in structure and sequence from other known cytokines, and the Gaffen lab has been a leader in studying signaling pathways mediated by this novel family of cytokines. In addition, antibodies against IL-17 and its receptor were recently approved by the FDA to treat autoimmune conditions, particularly psoriasis

The Gaffen lab studies three aspects of IL-17/Th17 cell biology: (1) mechanisms of molecular signal transduction mediated by IL-17 and its receptor (2) means by which IL-17 mediates host defense against mucosal Candida albicans fungal infections, and (3) mechanisms by which dysregulated IL-17/Th17 cells drive pathogenesis of autoimmunity.

Speaker: Kent Hill, PhD., Professor
Microbiology, Immunology, & Molecular Genetics
University of Los Angeles, California

Dynein Dragon Boat: Atomic Structure of the Trypanosome Axoneme Illuminates Mechanism of Flagellar Beating in Eukaryotes



Biography

Dr. Kent L. Hill is one of our very own SCEP veterans, with his lab joining this conference for more than a decade. He is a Professor in the Department of Microbiology, Immunology, and Molecular Genetics at the University of California, Los Angeles (UCLA). His research centers on the flagellar motility of African trypanosomes, the protozoan parasites responsible for African sleeping sickness. Dr. Hill investigates how these parasites use their flagella for movement and host interactions, aiming to understand the mechanisms of parasite motility and its implications for disease progression. His work has contributed to identifying key proteins involved in flagellum assembly and function, providing insights into potential therapeutic targets for trypanosome-related diseases.

SCEP Symposium Agenda

14th Annual SCEP Symposium

8:30-9:00am Breakfast and Registration (**SOM Education Building II, room 205, UCR**)
Poster set-up (**SOM Education Building II – Outdoor Terrace**)

9:00am-9:10 am **Welcome to the 14th annual SCEP symposium – Opening remarks**
Karine Le Roch, Ph.D.
Director Center for Infectious Disease and Vector Research, UC Riverside

9:10-9:45 am **SCEP Morning Speaker, introduced by Karine Le Roch**
Kent, Hill Ph.D.
Professor, Microbiology, Immunology, & Molecular Genetics, University of Los Angeles, California
Presentation title: Dynein Dragon Boat: Atomic Structure of the Trypanosome Axoneme Illuminates Mechanism of Flagellar Beating in Eukaryotes.

Morning Session

Chair: Dr. Rebecca Ruggiero-Ruff, Ph.D., Post-doctoral Fellow, UC Riverside

9:45 - 10:00 am: **Jennell Jennett,** Nair Lab, Biomedical Sciences, UCR School of Medicine
Title: Helminth-Induced Type 2 Immune Responses Protect Against Diet-Induced Obesity via RELMα.

10:00 - 10:15 am **Christina Hueschen, PhD,** Cell and Developmental Biology, UCSD
Title: Actin self-organization in gliding *Toxoplasma gondii*.

10:15 - 10:30 am **Shinhye Chloe Park,** Koshy Lab, University of Arizona
Title: The Role of a Hypothetical Protein (TGME49_207210) in *Toxoplasma gondii* Stage Conversion and Persistence.

10:30 - 10:45 am **Maura C Ruyechan,** Ralston Lab, Microbiology and Molecular Genetics, UC Davis.
Title: Trogocytosis of human cells by *Entamoeba histolytica* enables protection from human serum complement lysis

10 MINUTE STRETCH BREAK

10:55 - 11:10 am: **Johann Tailor,** Riestra Lab, Dept. of Biology, San Diego State University
Title: An Immunological Wildfire: *Trichomonas vaginalis* triggers pyroptosis in ectocervical cells

11:10 - 11:25 am: **Ruhi Patel, PhD,** Hallem Lab, Department of Microbiology, Immunology and Molecular Genetics, UCLA
Title: Dopamine signaling drives skin invasion by human-parasitic nematodes

Teaser talks

11:25 - 11:30 am: **Arrmund Neal,** Le Roch Lab, UCR
Title: Determining the role of PfCRWN-L protein in regulating chromatin structure in the human malaria parasite, *Plasmodium falciparum.*

11:30 - 11:35 am: **Arzu Ulu,** Wilson Lab, UCR
Title: Factors contributing to bradyzoite-to-bradyzoite replication following Toxoplasma gondii cyst recrudescence in murine primary astrocytes

11:35 - 11:40 am: **Kyle Anesko,** Dillman & Nair, Environmental Toxicology, UCR, Riverside
Title: Exploring novel immunomodulatory pathways between parasitic nematode excretory/secretory proteins and mammalian host-parasite interactions.

11:40 - 11:45 am: **Saarang Kashyap,** Zhou Lab, MIMG, University of California, Los Angeles
Title: Structures of Native Doublet Microtubules from Trichomonas vaginalis Reveal Parasite-Specific Proteins.

11:45 am - 12:00pm **BREAK and SQUEEZE UP!**

12:00-1:00pm **Keynote Speaker**, introduced by Meera Nair, PhD

Sarah L. Gaffen, PhD,

Gerald P. Rodnan Professor, Division of Rheumatology & Clinical Immunology, University of Pittsburgh.

Title: All Things Great and Seventeen: At the Crossroads of IL-17 Signaling in Fungal Immunity and Beyond.

1:00-2:30pm **Lunch and Poster Viewing (SOM educational Building - Patio)**

Afternoon Session

Chair: Karine Le Roch, Ph.D.

2:30 - 2:45 pm: **Isabel Romero,** Mercer lab, Biological Sciences Department and Cal Poly Pomona

Title: Investigating the role of antibodies and complement in neutrophil trogocytic killing of *Trichomonas vaginalis*

2:45 - 3:00 pm: **Brecken Enright,** Schulz Lab, Harvey Mudd College department of Biology

Title: Investigating the importance of chromatin interacting proteins in mediating transcription of procyclin genes during differentiation of African trypanosomes from the bloodstream to the procyclic form.

3:00 - 3:15 pm: **Juliette Uy,** Bradley Lab, Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles.

Title: Toxoplasma IMC1 is a central component of the IMC network and plays critical roles in parasite morphology, replication, and infectivity.

3:15 - 3:30 pm: **Angelica Lopez,** Jimenez Lab, Department of Biological Science, California State University, Fullerton.

Title: Study of an orphan kinesin reveals connections between the contractile vacuole and flagellar structures in Trypanosoma cruzi.

3:30 - 3:45 pm: **Katherine Yanes,** Lodoen Lab, Molecular Biology and Biochemistry, University of California, Irvine
Title: Toxoplasma gondii Drives Immune Cell Recruitment to Amyloid Plaques in Alzheimer's Mice.

3:45 - 4:00 pm: **Lauren Wong**, Buckley Lab, Biological Sciences, California Polytechnic State University, Pomona.
Title: The role of the Peripheral Cannabinoid Receptor on Neutrophil Egression into the Vagina.

4:00 - 4:15 pm: **Marco Martinez,** Lima Lab, California State Polytechnic University, Pomona
Title: Investigating the role of the innate immune system in entrapping and neutralizing Toxoplasma gondii by neutrophil extracellular traps (NETs).

4:15-6:00pm **Drinks and Nibbles (SOM Educational Building Patio)**
Poster presentations (odd numbers 4:15- 4:45pm; even numbers 4:45-5:30pm)
Announcement of abstract awards and networking (5:30-6:00pm)

Talk Abstract #1

Helminth-Induced Type 2 Immune Responses Protect Against Diet-Induced Obesity via RELMα

Jennell Jennett, Rebecca Ruggiero-Ruff, Kyle Anesko, Gary Chen, Yuxin He, and Meera G. Nair
UCR School of Medicine

Resistin -Like Molecule Alpha (RELMα), a macrophage-secreted protein, is crucial for immunomodulation and wound healing in type 2 immune responses, such as parasitic helminth infections. Obesity, the second leading cause of preventable death, exhibits a pro-inflammatory Th1 environment with M1 macrophages in visceral fat, while healthy adipose tissue has an anti-inflammatory Th2 environment with M2 macrophages. Our lab demonstrated that RELMα protects naive mice against diet-induced obesity via induction of M2 macrophages in visceral adipose tissue. We hypothesized that elevating macrophage-derived RELMα levels through helminth infection could protect from obesity and metabolic dysfunction. We employed two complementary methods to increase macrophage-derived RELMα: helminth infection and lentiviral-induced RELMα overexpression. Mice were fed a Western diet (high fat, high glucose) for 10–14 weeks to induce obesity followed by infection with Nippostrongylus brasiliensis. Weight was monitored, and glucose tolerance, cellular and cytokine changes, and histological responses were assessed three weeks post helminth infection. Myeloid-specific RELMα KO mice were utilized to determine myeloid cell-intrinsic RELMα effects. RELMα lentiviral overexpression in bone marrow-derived macrophages (BMDMs) allowed investigation of effects on macrophage polarization. Helminth infection-induced RELMα reduced weight gain, adipose mass, and adipocyte size, and improved glucose tolerance and anti-inflammatory changes in visceral fat. RELMα-overexpressing BMDMs exhibited enhanced responses to Th2 stimuli with increased M2 gene expression (arginase, chitinase-like3) and decreased Th1 responses with reduced M1 gene expression (TNFα). In conclusion, RELMα overexpression by macrophages may provide a promising strategy to treat diet-induced obesity and its associated comorbidities.

Talk Abstract #2

Actin self-organization in gliding Toxoplasma gondii

Christina Hueschen (UCSD); Segez-Zarko, Li-av (Stanford); Chen, Jian-Hua (UCSF); LeGros, Mark (UCSF); Larabell, Carolyn (UCSF); Boothroyd, John (Stanford); Phillips, Rob (Caltech); Dunn, Alex (Stanford)

During host infection, Toxoplasma gondii and related unicellular parasites move using gliding, which differs fundamentally from other known mechanisms of eukaryotic cell motility. Gliding is thought to be powered by a thin layer of flowing filamentous (F)-actin sandwiched between the plasma membrane and a myosin-covered inner membrane complex. How this surface actin layer drives the various gliding modes observed in experiments—helical, circular, twirling, patch, pendulum or rolling—is unclear. Here, we suggest that F-actin flows arise through self-organization and develop a continuum model of emergent F-actin flow within the confines provided by Toxoplasma geometry. In the presence of F-actin turnover, our model predicts the emergence of a steady-state mode in which actin transport is largely directed rearward. Removing F-actin turnover leads to actin patches that recirculate up and down the cell, which we observe experimentally for drug-stabilized actin bundles in live Toxoplasma gondii parasites. These distinct self-organized actin states can account for observed gliding modes, illustrating how different forms of gliding motility can emerge as an intrinsic consequence of the self-organizing properties of F-actin flow in a confined geometry.

Talk abstract #3

The Role of a Hypothetical Protein (TGME49_207210) in Toxoplasma gondii Stage

Shinhye C. Park, Chandrasekaran, Sambamurthy, Kochanowsky, Joshua A., Koshy, Anita A.

University of Arizona

Toxoplasma gondii is an obligate intracellular parasite that infects virtually all warm-blooded animals and causes the disease toxoplasmosis. Key to Toxoplasma's pathogenesis is its ability to differentiate from a fast-growing tachyzoite stage associated with acute infection to a relatively dormant bradyzoite stage contained in tissue cysts that are the hallmarks of a chronic infection. Encysted bradyzoites can reactivate to cause severe pathology in the immunocompromised and enable transmission between intermediate hosts. Given the importance of this stage, our lab seeks to define genes that enable stage conversion and persistence. This study focuses on a hypothetical gene (TGME49_207210) that we and others have identified as being highly upregulated in bradyzoites. TGME49_207210 codes for a protein of 110 amino acids and its only paralogs are found in other cyst forming protozoa. As nothing is known about the role or function of TGME49_207210, we knocked the gene out in a type II strain (IIΔ207210) and generated a FLAG-tagged complemented strain (IIΔ207210::207210). Using these strains, we determined that TGME49_207210 is only expressed as a bradyzoite and localizes to the cytosol. The IIΔ207210 strain shows a stage conversion defect in vitro and a persistence defect in vivo. As the next step, a highly active biotin ligase variant TurboID was fused to TGME49_207210 in a type II strain (II_207210_TurboID) to perform proximity labeling. Current work is focused on identifying protein binding partners and validating the in vivo work in a second cohort.

Talk Abstract #4

Trogocytosis of human cells by Entamoeba histolytica enables protection from human serum complement lysis

Maura C. Ruyechan, Huang, Wesley, Ralston, Katherine S

UC Davis

Entamoeba histolytica is the causative agent of amoebiasis. It uses the process of trogocytosis, or “cell nibbling”, that kills human cells contributing to tissue damage. Multiple eukaryotic cell types also perform trogocytosis. Notably, when immune cells nibble other cells, proteins from the nibbled cell are displayed on the immune cell surface. We found that E. histolytica displays human cell membrane proteins on its surface following trogocytosis, with associated protection from lysis by human serum complement. Therefore, we hypothesize that display of host proteins following trogocytosis directly prevents host complement activation on the amoebic surface. To determine if protection from lysis by complement is dependent upon this display, we allowed amoebae to perform trogocytosis on human cells and exposed them to mouse serum. Intriguingly, following trogocytosis of human cells, amoebae were protected from lysis by mouse serum. By exposing amoebae exogenously expressing negative regulators of human complement activation to mouse and human sera, we determined that mouse and human complement regulators are functionally interchangeable. We then allowed amoebae to nibble insect cells to determine if trogocytosis of cells without these complement regulatory proteins confers protection from lysis by human serum. The data show that following trogocytosis of these insect cells, no protection was conferred to amoebae, indicating that the display of host proteins is what confers protection to amoebae. The display of host proteins following trogocytosis is a novel mechanism for evasion of innate immune defenses. Since other pathogens also perform trogocytosis these studies should improve our understanding of virulence mechanisms.

Talk Abstract #5

An Immunological Wildfire: Trichomonas vaginalis triggers pyroptosis in ectocervical cells

Johann Tailor, (San Diego State University & UC San Diego), Howayer, Maryam (San Diego State University), Barouch, Evie (San Diego State University), Baxter, Bryn (San Diego State University & UC San Diego), Young, Brayden (San Diego State University), Adams, Michiko (San Diego State University), Riestra, Angelica (San Diego State University & UC San Diego)

Trichomonas vaginalis (Tv) causes the most common non-viral sexually transmitted infection. Increased inflammation upon Tv infection is predicted to help drive adverse health outcomes. Pyroptosis is an inflammatory cell death that is largely characterized in immune cells. We hypothesize that Tv damages ectocervical epithelial cells (Ect1) via pyroptosis. Pyroptosis involves caspase-1 cleavage of the Interleukin-1β (IL-1β) cytokine and the gasdermin D protein (GSDMD), which generates a gasdermin N-terminal fragment (GSDMD-NT). GSDMD-NT oligomerization forms pores on the cell membrane, prompting cytokine release and cellular rupture. To assess if Tv activates pyroptosis in ectocervical cells, we infected Ect1 cells and probed for gasdermin D processing via western blot. Tv-infection led to GSDMD-NT fragment generation in a parasite-burden manner. Next, we assessed the dynamics of Tv-induced host cell death using sytox green uptake and lactate dehydrogenase assays and found host cell damage occurring within 1hr of infection. Cell supernatant analysis utilizing ELISA revealed a statistically significant increase in IL-1β secretion from Tv-infected cells compared to uninfected Ect1 cells. Furthermore, the effect of inhibiting GSDMD-NT oligomerization using disulfiram was also tested. Disulfiram treatment led to a striking ~50% reduction in host cell lysis and IL-1β secretion compared to vehicle treatment during Tv infection. Lastly, using a genetic approach, we also observed a 25% decrease in cytolysis and a 53% decrease in IL-1β release in GSDMD knockout cells compared to WT amid infection. Collectively, our data suggest Tv triggers GSDMD-mediated pyroptosis in Ect1 cells and highlights the pivotal role of fiery pyroptosis during parasitic infection.

Talk Abstract #6

Dopamine signaling drives skin invasion by human-parasitic nematodes

Ruhi Patel, Garcia Romero, Aracely, Hallem, Elissa A.
University of California, Los Angeles

Skin-penetrating parasitic nematodes, including Strongyloides stercoralis, infect nearly 1 billion people worldwide, causing debilitating disease and fatalities. Skin penetration, the process whereby these nematodes invade host skin and enter the body, is a critical step that could be targeted to prevent infections. However, the behaviors that skin-penetrating nematodes execute during skin penetration and the underlying molecular mechanisms are poorly understood.

To first examine skin penetration, we established an ex vivo assay wherein S. stercoralis infective larvae are placed on excised rat skin and videorecorded. Larvae pushed down with their heads against the skin surface within seconds after contact. Thereafter, larvae either punctured the skin with their heads or crawled elsewhere. Some punctures led to complete penetration whereas others were aborted. Ultimately, larvae repeatedly pushed, punctured and crawled on skin until penetration was completed.

We next investigated the neural mechanisms that underlie skin penetration and hypothesized that the mechanosensory texture-sensing neurons might be necessary. The free-living nematode Caenorhabditis elegans senses texture using the dopaminergic neurons; thus, we inactivated dopamine signaling in S. stercoralis, by using the dopamine receptor antagonist haloperidol, and found that this inhibited skin penetration. Similar findings were obtained upon genetic inactivation of dopamine signaling using CRISPR/Cas9-mediated targeted mutagenesis. Excitingly, haloperidol also delayed or inhibited skin penetration in the human hookworm Ancylostoma ceylanicum. Thus, dopamine signaling likely plays a conserved role in driving skin penetration in multiple species of skin-penetrating helminths. Ultimately, our work could lead to the development of topical anthelmintics that prevent infections by inhibiting skin penetration.

Talk Abstract #7

Determining the role of PfCRWN-L protein in regulating chromatin structure in the human malaria parasite, P. falciparum

Arrmund Neal, Zeinab Chahine, Thomas Hollin, Charles Banks, Jacques Prudhomme, Laurence Florens, Karine G. Le Roch
University of California, Riverside

Malaria remains a global health threat with approximately 249 million cases and 608,000 deaths annually. Among the five species that infect humans, Plasmodium falciparum is the most virulent and is responsible for most malaria-related deaths. Its complex life cycle implies important gene regulation; however, our poor understanding of these mechanisms presents significant challenges to therapeutic interventions. Recent research from our lab has identified key chromatin-associated proteins, including the Crowded Nuclei (CRWN)-like protein (Pf3D7_1325400), which is known to regulate nuclear architecture in Arabidopsis, and is functionally like the lamina found in metazoans. In the absence of lamina in P. falciparum, we suspect CRWN plays a similar role, yet its function remains unclear. My project aims to use a functional genomic pipeline combining genome editing tools and a series of genome wide approaches to investigate the role of PfCRWN-L in the P. falciparum life cycle progression. We aim to uncover a novel parasite specific target for new therapeutic strategies against malaria.

Talk Abstract #8

Factors contributing to bradyzoite-to-bradyzoite replication following Toxoplasma gondii cyst recrudescence in murine primary astrocytes

Arzu Ulu, A. Leon, S. Morales, N. Kachour, K.V. Bergersen, E.A. Vizcarra, M.W. White, and E.H. Wilson
Wilson Lab — University of California, Riverside

Toxoplasma gondii, a foodborne intracellular parasite, affects about one-third of the global population, forming brain cysts that maintain chronic infection and lead to disease during reactivation. Despite its high prevalence, studying events post-cyst reactivation has been challenging due to the limited developmental capacity of the parasite in conventional cultures. To overcome this problem, we developed an ex vivo culture system, and identified a distinct pathway post-recrudescence, bradyzoite-to-bradyzoite pathway, avoiding a transitionary tachyzoite stage, that is dependent on host cell type and exclusive to primary murine astrocytes, not fibroblasts. To explore this host-cell dependence, we determined the need of bradyzoite-to-bradyzoite development on host-cell glucose content following recrudescence in astrocytes. We found that glucose availability influences this host-cell-dependent bradyzoite development. Low glucose levels promoted bradyzoite-to-tachyzoite replication, while high glucose supported bradyzoite replication by upregulating the bradyzoite antigen, SRS9. Manipulation of glucose levels using insulin or adrenaline demonstrated that insulin in high glucose expanded SRS9+ vacuoles, while adrenaline decreased their proportion. These findings indicate that a high-glucose environment supports bradyzoite-to-bradyzoite replication following cyst recrudescence. In summary, both host cell type and glucose availability play critical roles in the bradyzoite-to-bradyzoite developmental pathway. Our ongoing studies are focused on the molecular mechanisms driving this dependence.

Talk Abstract #9

Exploring novel immunomodulatory pathways between parasitic nematode excretory/secretory proteins and mammalian host-parasite interactions

Kyle Anesko, Yuxin He, Jennell Jennett, Pakeeza Azizpor, Akilma K. Lima, Meera Nair, & Adler R. Dillman

The University of California, Riverside

Host-parasite interactions represent a dynamic interplay between the immune system of the host and the strategies employed by parasites to establish infection. We investigate the role of excretory/secretory proteins (ESPs) released by parasitic nematodes, including Steinernema carpocapsae's ShK-domain (Sc-ShK-2 Domain) and Heligmosomoides polygyrus's FAR protein (Hp-FAR-1), in regulating host immune responses. By focusing on the interaction between nematode ESPs and mammalian immune cells, we aim to elucidate the mechanisms by which these proteins modulate immune cell function and to determine host specificity. Through immunoassays such as the LEGENDplex inflammation panel, we assess changes in cytokine profiles and immune cell activation in response to nematode ESPs. Furthermore, we explore the impact of nematode ESPs on the interaction between bone marrow derived macrophages (BMMacs) and Nippostrongylus brasiliensis (Nb) larvae. We anticipate that our findings will contribute to a more comprehensive understanding of host-parasite interactions, with implications for the development of novel therapeutic strategies targeting nematode-borne diseases. By elucidating the immunomodulatory role of nematode ESPs, our research opens new avenues for exploring the complexities of nematode pathogenesis and host immune evasion mechanisms.

Talk Abstract #10

Structures of Native Doublet Microtubules from Trichomonas vaginalis Reveal Parasite-Specific Protein

Saarang Kashyap, Stevens, Alexander, Crofut, Ethan H., Wang, Shuqi E., Muratore, Katherine A., Johnson, Patricia J., Zhou, Z. Hong
University of California, Los Angeles

Doublet microtubules (DMTs) are flagellar components required for the protist Trichomonas vaginalis (Tv) to swim through the human genitourinary tract to cause trichomoniasis, the most common non-viral sexually transmitted disease. Lack of DMT structures has prevented structure-guided drug design to manage Tv infection. Here, we determined the cryo-EM structure of native Tv-DMTs, identifying 30 unique proteins, including 19 microtubule inner proteins and 9 microtubule outer proteins. While the A-tubule is simplistic compared to DMTs of other organisms, the B-tubule features specialized, parasite-specific proteins, such as TvFAP40 and TvFAP35 that form filaments near the inner and outer junctions, respectively, that appear to stabilize DMTs and enable Tv locomotion. Notably, a small molecule, assigned as IP6, is coordinated within a pocket of TvFAP40 and has characteristics of a drug molecule. This first atomic model of the Tv-DMT highlights the diversity of eukaryotic motility machinery and provides a structural framework to inform rational design of therapeutics.

Talk Abstract #11

Investigating the role of antibodies and complement in neutrophil trogocytic killing of Trichomonas vaginalis

Isabel Romero, Trujillo, Emma, Flores, Barbara and Mercer Frances
Cal Poly Pomona

Trichomonas vaginalis is the causative agent of trichomoniasis, the third most common sexually transmitted infection in the United States. Neutrophils are the main immune cells found at the site of infection and defend against Trichomonas vaginalis using trogocytosis. Trogocytosis involves the neutrophils swarming the parasite and obtaining pieces from the parasite's plasma membrane, ultimately leading to death. The receptor-ligand interaction mediating trogocytosis is not characterized. Neutrophils must be in the presence of human serum to successfully kill the parasite. Serum factors include complement proteins and antibodies that could mediate parasite trogocytosis. The two major opsonic antibodies, IgA and IgG, can bind to neutrophils via Fc□R, Fc□RI, Fc□RIIa and Fc□RIII. However, the specific Fc receptor facilitating parasite killing is unclear. Moreover, Trichomonas vaginalis is opsonized by iC3b, a complement opsonin. Neutrophils express three receptors that bind to iC3b: complement receptor 1, complement receptor 3, and complement receptor 4. Here, we aim to use blocking antibodies to compare parasite killing when Fc and complement receptors are inhibited on primary neutrophils. The complement system also plays a role during the immune response against Trichomonas vaginalis. Mammals have adapted proteins that downregulate complement activity on self-cells called regulators of complement activation (RCAs). RCAs are plasmatic or membrane-bound inhibitory proteins necessary for the regulation of complement activation. However, pathogens have adapted mechanisms to avoid complement recognition by stealing host RCAs, mimicking host RCAs or blocking complement proteins involved in complement activation. It is unknown if Trichomonas vaginalis can acquire host RCAs.

Talk Abstract #12

Investigating the importance of chromatin interacting proteins in mediating transcription of procyclin genes during differentiation of African trypanosomes from the bloodstream to the procyclic form

Brecken Enright (Scripps College); Ela, Alessandra (Harvey Mudd College); Schulz, Danae (Harvey Mudd College)

Trypanosoma brucei, the causative agent of Human African Trypanosomiasis (HAT) and animal trypanosomiases, cycles between a bloodstream form in mammals and a procyclic form in the insect vector. In mammals the parasite evades the host immune system by antigenically varying its Variant Surface Glycoprotein (VSG). In flies, the parasite expresses one invariant procyclin protein from a limited number of EP and GPEET genes. The mechanism by which transcription of EP is initiated during differentiation is not well understood. We are using an EP1/GFP reporter system to screen chromatin interacting proteins that bind to transcription start sites for a role in surface protein remodeling. We first generate tet inducible RNAi lines for candidate genes in bloodstream parasites. We then differentiate these bloodstream parasites and look for differences in GFP expression as parasites transition from the bloodstream to the procyclic form. While knockdown of Hat1 and Set26 proteins had no discernible effect on EP1 expression, preliminary experiments indicate that knocking down the noncatalytic Eaf6 protein that is part of a NuA4 HAT complex in mammals causes a lag in GFP expression as parasites transition to the procylic parasite stage. In trypanosomes, Eaf6 was previously found to associate with both Bdf6 and Hat1 proteins in a complex that binds to transcription start sites; thus, it may be important for initiating transcription of the EP1 gene once the developmental signal to differentiate is received. We are currently generating FLAG-tagged Eaf6 lines so that we can accurately screen clones for robust knockdown. With these lines in hand, we will verify the lag in procyclin expression and perform assays to ascertain whether knockdown or deletion of Eaf6 compromises histone acetylation at the EP1 locus, which could compromise the parasite's ability to express EP1. We hope these experiments will lend insight into the mechanisms of transcriptional reprogramming as parasites transition between diverse host environments.

Talk Abstract #13

Toxoplasma IMC1 is a central component of the IMC network and plays critical roles in parasite morphology, replication, and infectivity

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The Inner Membrane Complex (IMC) is an apicomplexan-specific organelle that facilitates essential processes including replication, motility, and invasion. The IMC is composed of two distinct structures: a group of flattened peripheral vesicles called the IMC membrane and an underlying web of intermediate filament-like proteins that form the IMC network. The IMC network filaments are believed to be formed via the alveolins, a group of proteins characterized by a region of proline-valine repeats called the alveolin domain. IMC1 was the first alveolin identified and was thought to be essential, which has made its precise function elusive. In this study, we surprisingly were successfully able to disrupt IMC1 using CRISPR/Cas9, leading to an extreme defect in parasite morphology, invasion, and egress. Detergent extraction and fractionation experiments showed that the knockout parasites are far more fragile that wild-type strains. We then use deletion analyses and functional complementation to identify regions of IMC1 that are required for function and localization. These studies demonstrated that the C-terminal region of the alveolin domain is critical for protein function, while the N-terminal region, which contains multiple palmitoylation sites, is dispensable. We additionally conducted in vivo assays and found the knockout parasites were unable to establish a chronic infection. To examine interactions with other alveolins, we conducted pairwise yeast 2-hybrid assays against other IMC alveolins in the network. Overall, our findings provide new insights into the role of IMC1, solidifying its importance as a key component of the IMC network.

Talk abstract #14

Study of an orphan kinesin reveals connections between the contractile vacuole and flagellar structures in Trypanosoma cruzi

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The contractile vacuole complex (CVC) of Trypanosoma cruzi is a bipartite organelle that regulates cell volume. Water and osmolytes are collected in the bladder of the CVC and expelled to the flagellar pocket. The filling state of the bladder is sensed by a mechanosensitive channel (TcMscS) required for normal function. Beyond its osmoregulatory function, the CVC has newly revealed roles related to trafficking of flagellar and surface proteins, but the mechanisms regulating protein distribution has not been characterized. We have found that mechano-deficient parasites lacking TcMscS, have important defects in cytokinesis and flagellar formation. In these parasites, an orphan kinesin with homology to the Kinesin of the Ingressing Furrow (TcKLIF) is significantly downregulated. We sought to establish the expression and localization of this protein in the three life stages of T. cruzi and found a robust expression in the flagellar attachment zone of epimastigotes and trypomastigotes. Analysis of cell division in synchronized parasites showed important differences in the localization of TcKLIF between cells in G1 and G2. Since the ortholog in T. brucei (TbKLIF) plays an important role in cytokinesis, we knocked out TcKLIF by CRISPR-Cas9 gene targeting and found that, while the protein is not essential, its ablation results in growth defects, abnormal kinetoplast/nuclei ratio and cell cycle dysregulation. The TcKLIF-KO parasites also showed impaired differentiation and significantly reduced infectivity. Our results suggest that TcKLIF may be playing an important role in maintaining flagellar structures and repositioning of organelles required for parasite division and life stage transition.

Talk Abstract #15

Toxoplasma gondii Drives Immune Cell Recruitment to Amyloid Plaques in Alzheimer's Mice

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Background: Toxoplasma gondii is an intracellular foodborne parasite infecting approximately 30% of the global population. T. gondii infects the host's brain, where it induces a neuroimmune response. In Alzheimer's model mice, T. gondii infection is known to result in amyloid plaque reduction, but it is unclear whether peripheral cells are recruited to plaques during infection and by what mechanism.
Hypothesis: T. gondii infection recruits peripheral immune cells to amyloid plaques in the brain, contributing to plaque reduction.
Methods: 3-month-old 5xFAD (AD model) mice were injected with 200 T. gondii tachyzoites or PBS (control). After 2-6 weeks, brains were harvested and analyzed by flow cytometry and microscopy to characterize infiltrating immune cells. The extent of peripheral immune cell localization to plaques was assessed using 5xFAD bone marrow chimeric mice. Infection of 5xFAD TREM2 KO mice was used to determine the role of TREM2 in these processes.
Results: T. gondii infection reduced amyloid plaque volume in 5xFAD mice, whereas colocalization of amyloid with myeloid cells and CD68 (phagocytic marker) increased after infection. Bone marrow chimera experiments demonstrated that infection recruited peripheral immune cells into the brain and near plaques. TREM2 may be required for increased myeloid cells surrounding plaques but not for immune cell recruitment to the brain during T. gondii infection of 5xFAD mice.
Conclusions: T. gondii infection reduced amyloid plaques, partly due to the recruitment of peripherally derived immune cells and activated microglia near plaques. Future studies will focus on the effect of T. gondii infection in human AD patients.

Talk Abstract #16

The role of the Peripheral Cannabinoid Receptor on Neutrophil Egression into the Vagina

Lauren Wong and Nancy Buckley

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Vulvovaginal candidiasis (VVC) is an infection primarily caused by an overgrowth of Candida albicans (C. albicans). About 75% of women will experience VVC at least once in their lifetime. Susceptibility to VVC depends on factors such as sexual activity, immune status, and use of immune suppressive drugs such as marijuana. Delta-9- tetrahydrocannabinol (THC), the primary psychotropic compound in marijuana, binds to cannabinoid receptors such as the peripheral cannabinoid receptor (CB2R) which is primarily found on immune cells. Neutrophils are the primary innate immune cell to fight VVC that could be affected by THC. We first investigated the role of CB2R in VVC. Wild type (CB2R+/+) and CB2R knockout (CB2R-/-) female mice were injected subcutaneously with estradiol, three days prior to infection with 5x106 C. albicans yeast cells/mL. The vagina, vaginal lavage (VL) and bone marrow (BM) cells were collected 4 days after the infection. Vaginal fungal load was determined by counting C. albicans colony forming units. VL and BM cells were analyzed via flow cytometry to assess neutrophil levels. BM cell number is comparable between CB2+/+ and CB2R-/-. VL cell number is 3 times less in CB2R-/- compared to CB2R+/+ in uninfected mice and 12 times less in CB2R-/- compared to CB2R+/+ in infected mice. The BM neutrophil CB2R+/+/CB2R-/- ratio was 1 in uninfected mice and 3 in infected mice. The VL neutrophil CB2R+/+/CB2R-/- ratio s was 11 in uninfected mice and 28 in infected mice. These data suggest that CB2R may impair egression of neutrophils into the vagina.

Talk Abstract #17

Investigating the role of the innate immune system in entrapping and neutralizing Toxoplasma gondii by neutrophil extracellular traps (NETs)

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Approximately one-third of the global human population harbors an infection with the protozoan parasite Toxoplasma gondii. The immune system relies on neutrophils and possibly one of their key mechanisms, neutrophil extracellular traps (NETs), to entrap and neutralize T. gondii. NETs are comprised of DNA, myeloperoxidase, citrullinated-histone 3, and elastase. Traditionally, NET release was thought to result in neutrophil cell death. However, recent findings have suggested an alternative outcome - vital NETosis, which allows NET formation while preserving cell viability. While T. gondii induces NETs, the question remains whether these are released through vital NETosis. Additionally, the cellular interactions and signaling that trigger vital NETosis are unknown. Here, the promyelocytic cell line HL-60 was differentiated into neutrophil-like cells (NLCs) using retinoic acid and DMSO. NLCs or primary human neutrophils were then infected with T. gondii at a multiplicity of infection of 2 in the presence of SYTOX, a dye that binds to extracellular DNA. Fluorescence was quantified with a plate reader and compared with PMA-stimulated cells, an inducer of suicidal NETosis. Results showcased an increase in extracellular DNA in T. gondii-infected NLCs and primary human neutrophils. Immunofluorescence microscopy at various time points confirmed NET formation. The viability of T. gondii-infected NLC at later time points was confirmed by zombie staining and measurement of extracellular ATP. The use of a RAB27a inhibitor, Nexinhib20, suggested a role for this vesicle trafficking protein in T. gondii-induced NETosis. The next stages of this project are determining whether neutrophil complement receptors mediate T. gondii-induced vital NETosis and if the role of RAB27a is associated with vital NETosis.

Poster Abstracts

1. Examining the role of degranulation in neutrophil trogocytic killing of Trichomonas vaginalis

Arielle Angel, Suhani B. Bhakta, Bethany N. Sesti, and Frances Mercer
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Neutrophil exocytosis of toxic granules (degranulation) is one method used to kill invading microorganisms. It may have a role in the contact-dependent killing of a protozoan parasite known as Trichomonas vaginalis. T. vaginalis is the causative agent of trichomoniasis, the third most common sexually transmitted infection in the United States. Neutrophils can kill T. vaginalis, but the mechanism is still poorly understood. Neutrophils cannot survive more than a day, but a progenitor cell line, HL-60, has been shown to be an effective alternative to neutrophils in vitro assays. These HL-60 cells can be differentiated into neutrophil-like cells (NLCs) and then stimulated to degranulate. During granulopoiesis, the development of granulocytes, granule formation in neutrophils occurs sequentially, starting with primary granules, then secondary and tertiary granules, and finally, secretory vesicles. However, the release of these granules occurs in the reverse order: secretory vesicles are released first, followed by tertiary, secondary, and then primary granules. The gene RAB27A is associated with the release of neutrophil's toxic granules. By knocking out RAB27A using CRISPR Cas 9, we expect these cells to no longer degranulate. Knockout of RAB27A was confirmed by sequencing, detecting exocytosis of primary granules, and measuring myeloperoxidase (MPO) secretion. Primary granules are the most toxic and exocytosis of these granules can be detected with the upregulation of a marker found on the surface of primary granules called CD63. We will use our RAB27A knockout cells to determine if the exocytosis of neutrophils assists in killing T. vaginalis. Similarly, an alternative route to test this process is using a chemical inhibitor known to inhibit exocytosis in neutrophils. Nexinhib20, a small molecule, can inhibit the Rab27a – JFC1 binding and impair neutrophil exocytosis. We plan to incubate Nexinhib20 with primary neutrophils and investigate if degranulation is inhibited. Therefore, with these two approaches, we will be able to determine if neutrophil exocytosis assists in the killing of T. vaginalis and further our knowledge of the mechanism of the killing of neutrophils by this protozoan parasite.

2. Investigating the role of motility in Trichomonas vaginalis pathogenesis

Bryn Baxter and Angelica Riestra
Riestra Lab — San Diego State University

Trichomonas vaginalis is a protozoan parasite responsible for the most common non-viral sexually transmitted infection, trichomoniasis. Trichomoniasis affects nearly 156 million people each year. Trichomoniasis is associated with adverse health outcomes including a greater risk of preterm birth, cervical and prostate cancer, as well as an increased risk of acquiring HIV. Although T. vaginalis poses a public health threat, the molecular mechanisms underlying infection remain understudied. Interestingly, the parasite has a unique characteristic of having five flagella which give rise to its striking motility. We hypothesize that T. vaginalis motility plays a key role in mediating infection. To investigate this, we performed the first characterization of kinesin-2 proteins in T. vaginalis. Kinesin-2 proteins are motor proteins that mediate anterograde intraflagellar transport, a process critical for flagellar assembly and maintenance. To determine the cellular localization of these putative flagellar proteins, we generated fusion protein constructs of the candidate kinesin-2 genes with two C-terminal HA tags and mNeonGreen, a green fluorescent protein. We then transfected T. vaginalis with these constructs using nucleofection technology. Using confocal microscopy, we found that two kinesin-2 fusion proteins localize to the flagella, cell membrane, and axostyle. We are currently optimizing CRISPR/Cas9 methodology to knockout these kinesin-2 genes, aiming to generate the first motility mutants in T. vaginalis. An update on these research efforts will be presented. Our work will generate novel knowledge about the cellular biology of T. vaginalis flagella and the contribution of T. vaginalis motility towards pathogenesis.

3. Determining the response of the protozoan parasite Tritrichomonas foetus to bovine neutrophils

Michael Berry and Frances Mercer
Mercer Lab — California State Polytechnic University, Pomona

Bovine neutrophils kill the protozoan parasite Tritrichomonas foetus using a mechanism known as trogocytosis, which involves the removal of fragments of the parasite membrane. Tritrichomonas foetus is the causative agent of trichomonosis, a recurring sexually transmitted infection that occurs in cattle and can cause spontaneous abortion in pregnant cows. Though it is known that bovine neutrophils kill T. foetus parasites by trogocytosis, the mode of death of the parasites following trogocytosis is unknown. We plan to test if the parasite undergoes apoptosis or lysis after neutrophil trogocytosis. In the closely related protozoan parasite T. vaginalis, there is variability in parasite strain resistance to human neutrophil killing by trogocytosis. Whether there is variability in bovine neutrophil killing of T. foetus is unknown. The T. foetus parasite strain KV-1 used previously to study bovine neutrophil killing will serve as the baseline to compare two other T. foetus strains, BP-4 and Belfast, resistance to neutrophil killing. So far, Belfast has proved to be more resistant than KV-1 under certain culture conditions. The mechanism by which different T. vaginalis parasite strains are more resistant than others is unknown, however a current hypothesis is that the resistant parasite strains are more effective at membrane repair, a known mechanism of cell survival in mammalian cells. We plan to compare resistant strains of T. foetus to the susceptible strains in their ability to repair their membranes. These studies will further our knowledge of the bovine neutrophil trogocytic response to Tritrichomonas foetus, and may aid in understanding the weak bovine immune response to T. foetus.

4. The Toxoplasma gondii alveolin proteins form a highly interconnected structure in the inner membrane complex

Samuel J. Choi and Peter J. Bradley
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Apicomplexan parasites possess several specialized structures, such as the inner membrane complex (IMC) for successful invasion and replication. The IMC is a conserved membrane-cytoskeletal system composed of a series of flattened vesicles supported by a subpellicular network (SPN) beneath the cell membrane. In Toxoplasma gondii, the SPN includes fourteen intermediate filament-like proteins called alveolins which are characterized by a conserved alveolin domain and play roles in reinforcing the organelle and assisting in daughter cell assembly. Here we identify two alveolin subcomplexes: IMC7 and IMC12 form an interdependent complex exclusive to maternal parasites, while IMC3, IMC6, and IMC10 form a complex enriched in the daughter IMC. Utilizing CRISPR gene knockout and pairwise yeast-two-hybrid (Y2H) assays, we demonstrate direct binding between IMC7 and IMC12. Previous work indicated IMC3 and IMC6 interact via their alveolin domains; our Y2H analysis further reveals an interaction between IMC10 and both IMC3 and IMC6. AlphaFold prediction suggests that the alveolins utilize beta sheets formed via the alveolin domain for interaction and filament formation. However, truncation studies of IMC10 shows that the minimal functional region surprisingly lies outside the alveolin domain in the C-terminus of the protein, which includes a valine rich region that appears to be sufficient for tethering to the IMC. These findings make IMC10 the first alveolin shown to function without its alveolin domain. While the IMC7-IMC12 complex supports the model of alveolin domain interactions and filament formation, evidence from IMC10's C-terminal binding suggests presence of uncharacterized protein features for SPN localization and filament assembly.

5. Host-cell dependent epigenetic profiles associated with differential expression and survival outcomes for unadapted T. gondii parasites

Loic Ciampossin, Arzu Ulu, Todd Lenz, Steven Abel, Sandeep Srivastava, Emma Wilson, Michael White, and Karine Le Roch
Le Roch Lab — University of California, Riverside

The recrudescence of Toxoplasma cysts remains a potentially lethal outcome of toxoplasmosis in immunocompromised individuals. Historically, Toxoplasma has been invaluable as a parasite model due to its ease of genetic modification and culturing, but the study of recrudescence from tissue cysts has been complicated by cell culture-adapted strains producing low-yield cysts. Using a novel ex vivo model with a Type II ME49 strain unadapted to fibroblast cell culture, we investigated the epigenetic mechanisms underlying these processes through ChIP-seq and examined their transcriptional outcomes with RNA-seq. Our study explores how the global epigenomic landscape and gene expression profiles differ between parasites grown in astrocytes (AST) and fibroblasts (HFF), revealing distinct host-cell-dependent effects on parasite invasion, growth, and replication. Comparative analyses between the two environments show a clear divergence in parasite population dynamics, which coincides with a reduction in the H3K4me3 activating mark at promoter regions over time in HFF-hosted parasites. This epigenetic outcome aligns with transcriptomic changes in cell cycle progression, growth, and development genes, including a subset of AP2 transcription factors, highlighting how the host environment influences parasite biology and developmental transitions.

6. Dynamics in trichomoniasis infection: Trogocytosis, efferocytosis and polarization

Ximena Corona, Suhani B. Bhakta, Bethany N. Sesti, and Frances Mercer
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Trichomonad infections by Trichomonas vaginalis (Tv.) remain poorly understood, despite infecting 3-10 million Americans annually. Left untreated, trichomoniasis has been associated with adverse reproductive complications including miscarriage, infertility and preterm labor. At the site of infection, neutrophils use a novel killing mechanism, trogocytosis, in which they trogocytose the parasite, taking bites of the plasma membrane, rather than engulfing. The role of neutrophil degranulation during this trogocytic response has not been described. Syntaxin Binding Protein 2 (STXBP2) facilitates degranulation by clasping receptors from the granule vesicle with receptors on the neutrophil plasma membrane. This fuses the granule, expelling the contents to the extracellular space, where we hypothesize that granule contents may degrade Tv's surface, potentially aiding the neutrophils in taking bites. We hypothesize knock-out STXBP2 neutrophils will have a reduced ability to trogocytose Tv. To test this, we will perform STXBP2 loss-of-function experiments introducing the degranulation-deficient neutrophils to Tv in co-culture assays, to assess the impact on trogocytic killing. Furthermore, it is not yet known if macrophages efferocytose (engulf) and polarize into M1 or M2-like macrophage subclasses following neutrophil trogocytosis. Balanced M1:M2 ratios are essential, as a harsh skew in either direction may have adverse consequences depending on the specific context of the infection. We hypothesize that macrophages efferocytose neutrophil-parasite complexes, polarizing into high M1:M2 subclass ratios. How neutrophils die following trogocytosis may also influence the predominating macrophage subclass. Therefore, we will utilize co-culture experiments with neutrophils, trichomonads, and macrophages to determine (a) how neutrophils die following trogocytic killing, (b) if macrophages engulf dead neutrophil-parasite complexes, and (c) what M1: M2 macrophage ratio emerges. Understanding these immune mechanisms is crucial for vaccine development especially as metronidazole resistance occurs in 4%-10% of vaginal infections. Moreover, studying polarization ratios may lead to novel therapeutics targeting macrophage subclasses, as seen in other biopharmaceuticals.

7. TREM2 is a critical regulator of the immune response against acute Toxoplasma gondii infection

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Monocytes, neutrophils, and macrophages form the first line of defense against pathogens. It is crucial that these cells be tightly regulated to allow for optimal infection control but also ensure against excessive inflammation and host survival. The Triggering receptor expressed on myeloid cells 2 (TREM2) receptor has been well-studied as an important rheostat of inflammation and function, but its effects on the immune system depend strongly on the type of pathogen. We investigated the role of TREM2 in the context of acute Toxoplasma gondii infection, a timepoint where the innate response is most important for parasite control. We infected TREM2-deficient “KO” and “WT” C57Bl/6 mice with low doses of a moderately virulent strain of the parasite. We found an early deficiency in chemotaxis and innate cell infiltration into the site of infection at 3 dpi and decreased functionality of these cells. This is correlated with an increased parasite burden at 7 dpi, as well as increased inflammation, pathology, and mortality in the knockout animals. These data show a clear link between the role of TREM2 in mounting a well-regulated, functional early innate response and later parasite clearance and host survival.

8. pH and the symbiont Mycoplasma hominis impact on Trichomonas vaginalis host-parasite interactions

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Trichomonas vaginalis (T. vaginalis) is the agent of the most prevalent non-viral sexually transmitted infection worldwide. This parasite resides in the human vaginal tract with the normal ranges in pH between 3.8- 5 in women of reproductive age. In contrast, the vaginal pH of healthy postmenopausal women is typically equal to or greater than pH 5.5. Approximately 45% of T. vaginalis clinical isolates host the bacterial symbiont Mycoplasma hominis (Mh). There is limited information on the impact of Mh on T. vaginalis infection and nothing is known about the effect of low pH on the T. vaginalis/Mh symbiosis and how this might affect the interaction of T. vaginalis with host cells. To address the effects of both pH and Mh on T. vaginalis infection, we have compared both the adherence to and cytolysis of host epithelial cells by the parasite at different pHs and in the presence and the absence of Mh. We found that lower pH in the presence of Mh increases host cell adherence and cytolysis. To identify parasite proteins with altered expression that may mediate the different pathogenic phenotypes conferred by pH and Mh, we have also conducted RNA-seq and proteomic analyses. The results of these analyses and their likely effect on pathogenesis will be discussed.

9. Toxoplasma gondii and the brain: Understanding the role of chronic toxoplasmosis in altering cognition and memory due to changes in glutamate transporter 1 (GLT-1)

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Toxoplasma gondii is an intracellular parasite infecting approximately one-third of the global human population. T. gondii is severely neglected and underrepresented in science and medicine. Although most acute infections are mild or asymptomatic, T. gondii crosses the blood-brain barrier, establishing chronic infection in the brain. Recent evidence indicates that chronic T. gondii infection decreases expression of astrocytic glutamate transporter (GLT-1), subsequently inducing increased extracellular glutamate. Excessive extracellular glutamate is neurotoxic and leads to neuronal damage. Recent literature has shown that T. gondii expresses parasite virulence factors that are secreted into the host cell and dysregulates NF-κB signaling, the main transcription factor for GLT-1. In vitro we have shown, in astrocytes, a decrease in GLT-1 and NF-κB expression 3 days post infection (dpi). Additionally, we have established parasite differentiation from T. gondii tachyzoites (acute infection) to bradyzoites (chronic infection) using neural progenitor stem cell line NE4C, infected with type II T. gondii (Pa-7 GFP) tachyzoites at a multiplicity of infection (MOI) 1. Bradyzoite differentiation was induced by maintaining the culture under alkaline stress. The frontal lobe and hippocampus are important brain regions for cognition and memory respectively. In vivo, using frontal lobe whole cell lysates, we detected a decrease in GLT-1 expression. In the hippocampus, there were no changes in GLT-1 expression. To assess change in cognition and memory, C57BL/6 mice are placed in the Barnes maze at 0 and 28 dpi. We have shown at 28 dpi there is a significant decline in cognition but not in memory.

10. Two G-type lectin receptor kinases regulate tomato reproduction and immunity against root-knot nematodes

Damaris Godinez-Vidal, Khanh Huynh, Megan Han, Han Hsi Chiu, Mohamed Ali, Isgouhi Kaloshian, and Simon C. Groen
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Plants and their parasitic nematodes are in a continuous co-evolutionary struggle for dominance. Consequently, plants have evolved strategies to perceive invading parasites and pathogens by receptors that recognize conserved molecular patterns of the invaders to induce pattern-triggered immunity. Previously, we found that root-knot nematode (RKN) perception and early responses in plants were similar to those of microbial pathogens and required the BRI1-ASSOCIATED KINASE1/SOMATIC EMBRYOGENESIS RECEPTOR KINASE3 (BAK1/SERK3) coreceptor in tomato (Solanum lycopersicum) and Arabidopsis (Arabidopsis thaliana). We also identified the transmembrane receptor–like kinase ENHANCED RESISTANCE TO NEMATODES1 (ERN1), a G-type lectin receptor kinase (G-LecRK), as an additional receptor involved in this process in Arabidopsis. To identify if the role of ERN1 in regulating resistance or sensitivity to RKNs is conserved in tomato, we employed CRISPR/Cas9 gene editing to knock out the two tomato paralogs of Arabidopsis ERN1. Although we did not observe differences in root and shoot growth, flowering, and seed set between wild-type and heterozygous mutant plants, homozygous mutants experienced lethality. Heterozygous mutants did show increased susceptibility to the RKN Meloidogyne incognita infection. To verify if homozygosity of the knock-out mutations also enhances plant susceptibility, we used a tomato hairy root system and knocked out both paralogs via CRISPR/Cas9 gene editing with the same construct as before. Roots homozygous for knock-out mutations in both G-LecRK genes showed higher susceptibility to M. incognita infection. Taken together, our findings suggest a rewiring of the regulatory networks underpinning plant reproduction and immunity between Arabidopsis and tomato and that G-LecRKs are essential for both processes in tomato.

11. Investigating the role for Bromodomain Protein 3 in initiating transcription of procyclin genes during differentiation of African trypanosomes from the bloodstream to the procycic form

Ethan Goroza, Ashley Tan, Evan Kim, Alessandra Ela, Jolyne Lin, Kieran Saucedo, Melvin Hodanu, and Danae Schulz
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Trypanosoma brucei, the causative agent of Human African Trypanosomiasis (HAT) and animal trypanosomiases, cycles between a bloodstream form in mammals and a procyclic form in the insect vector. In mammals the parasite evades the host immune system by antigenically varying its Variant Surface Glycoprotein (VSG). In flies, the parasite expresses one invariant procyclin protein from a limited number of EP and GPEET genes. The mechanism by which transcription of EP is initiated during differentiation is not well understood. We previously showed that occupancy of the bromodomain protein Bdf3 binds to acetylated histones at transcription start sites for the EP and GPEET regions of the genome. To investigate whether Bdf3 is sufficient for activating transcription at the EP locus, we are using a CRISPR activation system to tether Bdf3 to the EP locus using stable guide RNAs, and employing this system in bloodstream parasites that should not normally transcribe this procyclin gene. Using a GFP reporter system, we used flow cytometry to quantify the amount of EP transcription in the presence of a tethered Bdf3-Cas9 fusion protein. Preliminary experiments indicated an issue that the parent cell line that was not transfected with the stable guide RNAs was expressing a noticeable background level of GFP, and to address this, we used single cell cloning to isolate a parent cell clone that expressed a low level of GFP. Understanding the mechanisms of gene regulation in this highly diverged eukaryote may lend insight into how mechanisms of gene regulation evolved across diverse biological systems.

12. Functional Characteristics of Temperature Sensitive Mutants in Toxoplasma gondii

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Toxoplasma gondii is an obligate intracellular parasite with complex mechanisms for survival and proliferation. This study investigates two temperature-sensitive T. gondii mutants, 10-73C1 (mutant #6) and 12-88A5 (mutant #23), each exhibiting specific defects in proteins essential to the parasite's cell cycle. Notably, both mutants were classified as G1 phase-defective based on genomic DNA content measurements and the use of cell cycle markers. These mutants were obtained through a large chemical mutagenesis screen involving more than 60,000 individual clones; mutants conditional lethal at high temperature were obtained at a 0.26% overall frequency. Genetic rescue using fosmid/cosmid recombination and/or whole genome sequencing were used to identify the defective proteins in these mutants. Mutant 10-73C1 harbors a defect in a HEAT-containing protein, while 12-88A5 is defective in Yip1A, a presumed Golgi/ER membrane protein. Preliminary results reveal that both proteins are indispensable for cell cycle progression and survival, with the HEAT-containing protein appearing particularly crucial for maintaining viability. Further studies will confirm the mutations are responsible for temperature lethality and using epitope-tagging methods we will characterize the expression and intracellular location of each protein.

13. Investigating the role of pyroptosis in Trichomonas vaginalis infection in ectocervical cells

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Trichomonas vaginalis (Tv) is a sexually transmitted eukaryotic parasite that causes trichomoniasis. Women bear the comorbidities associated with Tv infection such as preterm birth. Pyroptosis is a pro-inflammatory form of cell death triggered by inflammasome activation leading to caspase-1 activation. Active caspase-1 in turn cleaves the gasdermin D protein and the gasdermin D N-terminal (GSDMD-NT) cleavage fragment forms membrane pores. We hypothesize that Tv kills ectocervical cells through pyroptosis. We investigated the contribution of caspase-1 towards killing an ectocervical cell line (Ect1) by treatment of cells with Ac-YVAD-cmk, a peptide-based caspase-1 inhibitor, and assaying for the release of lactate dehydrogenase (LDH) arising from cell membrane injury. A dose-dependent decrease in LDH release was observed upon caspase-1 inhibition compared to vehicle treatment. To further test if Ect1 cell killing occurs via pyroptosis, Ect1 infection was performed in the presence of disulfiram, a drug that blocks oligomerization of the cleavage fragment. Disulfiram treatment led to a striking 87% reduction in host cell lysis compared to GSDMD-NT vehicle treatment. Lastly, to identify which inflammasome complex contributes to the caspase-1 activation and pyroptotic cell we observed Ect1 exposure to Tv, Ect1 cells were infected in the presence of the AIM2 inflammasome inhibitor ODN-A151 and IL-1β release into cell supernatants was quantified by ELISA. AIM-2 inflammasome inhibition led to a 58% decrease in IL-1β secretion by Tv-infected cells compared to infected cells treated with vehicle control. Overall, our findings highlight that AIM2 inflammasome activation and pyroptosis contribute to the host response mounted against T. vaginalis.

14. Long-acting malaria prophylaxis via antisense oligonucleotides

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Malaria continues to impose a severe global health burden, largely due to the lack of effective vaccines and long-acting prophylactics. Anti-sense oligonucleotides (ASOs) present a promising solution to address this issue, offering several advantages over existing vaccine and chemoprophylactic approaches. ASO backbones may be chemically modified to increase its stability and half-life, and other known modifications and conjugations can enhance binding affinity, thermostability, and tissue selectivity. By acting at the mRNA level, ASOs can also target genes that have been previously undruggable using small molecule approaches and thus could greatly increase the number of potential effective antimalarial drug targets. Despite such promising properties, these ASO modifications have yet to have been evaluated in Plasmodium falciparum, and its efficacy in vivo unevaluated. Here, we screened 192 ASOs with 13 different chemical modifications against 5 different targets in blood-stage and liver-stage of Plasmodium infection. With our most potent liver-stage ASO, we performed chemoprophylactic treatment in a mouse model and demonstrated robust prevention from sporozoite infection in a dose-dependent manner. Our findings demonstrate that ASOs can be developed as an effective chemoprophylactic modality for malaria.

15. Investigating the underlying mechanisms of decreased GLT-1 expression during chronic Toxoplasma Gondii infection

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Toxoplasma gondii is an intracellular parasite that affects over 40 million people in the United States. The parasite causes infection in long lived tissue cells such as the heart, brain, and muscles. T. gondii crosses the blood barrier, where it can then differentiate from a stage one tachyzoite into a stage two bradyzoite, forming a chronic infection in the brain. Studies show that in the presence of a T. gondii infection, there is a decrease in the expression of glutamate transporter (GLT-1) in astrocytes, leading to excess glutamate in the synaptic cleft, causing excitoxicity. There has been recent data indicating that T. gondii releases unknown factors into the synaptic cleft, dysregulating NF- κB expression, which is the primary transcription factor for GLT-1. Here, we aimed to investigate NF-κB expression and activation in T. gondii-infected astrocytes. So far, we differentiated T. gondii tachyzoites (acute infection) into bradyzoites (chronic infection) using neural progenitor stem cell line NE4C infected with type II T. gondii (Pa-7 GFP) tachyzoites at a multiplicity of infection (MOI) 1. Bradyzoite differentiation was induced by maintaining the culture under stressful conditions (alkaline pH and low CO2). Our in vitro studies have shown that during chronic T. gondii infection, total expression of NF-kB was inhibited in astrocytes. Additionally, we did not find astrocytes-containing bradyzoites in our cultures, which suggests that infected neurons likely play an indirect role in inhibiting NF-κB signaling in astrocytes. Our future experiments aim to confirm our findings in chronically infected C57BL/6 mice brains.

16. Investigating the role of Mycoplasma hominis on the adherence of Trichomonas vaginalis to host cells

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Trichomonas vaginalis is responsible for one of the most prevalent sexually transmitted infections globally. Despite the prevalence of the infection, the mechanism driving many host-parasite interactions remain relatively unexplored. It has been established that the parasite's capacity to adhere to host cells, and subsequent cytolysis of the host cell, is an important factor in its ability to both establish and sustain an infection. The pathogenicity of T. vaginalis varies among strains, influenced by factors like the excretion of extracellular vesicles from the parasite and parasite surface proteins. This study explores how Mycoplasma hominis, a bacterial symbiont found in many T. vaginalis strains, enhances the parasite's pathogenicity by increasing adherence to host cells and altering gene expression of the parasite. Using transgenic T. vaginalis strains with genes upregulated in M. hominis-positive conditions, we assess the symbiont's impact on parasite adhesion and pathogenesis.

17. Bradyzoite replication is supported by skeletal myocytes, but not gut epithelial cells post cyst recrudescence

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About one third of the human population is estimated to be chronically infected with T. gondii. Populations with a compromised immune system, including patients with primary or acquired immunodeficiencies are vulnerable to developing lethal toxoplasmosis. While some therapies may be able to control acute toxoplasmosis, no therapies have been developed to eliminate chronic infection, manifested by drug-resistant cysts that primarily form in the brain. This is in part due to our limited understanding of the parasite's developmental pathway closely following recrudescence (reactivation) of the latent stage. We recently demonstrated that cyst-forming bradyzoites have at least two differentiation pathways following cyst recrudescence. They can follow the canonical pathway to generate fast growing, cell-lytic tachyzoites, or they can divide as bradyzoites and form new cysts—the 'brady-brady' pathway. Furthermore, the brady-brady pathway occurs only in specific cell types, thus this replication takes place in CNS resident astrocytes but not in fibroblasts. In the current study, we wished to determine if the brady-brady pathway occurs in skeletal myocytes (another common host of T. gondii cysts) and gut epithelial cells (involved in initial parasite dissemination). We used flow cytometry and immunofluorescence to assess the quantity and replication of tachyzoite (SAG1+) versus bradyzoite (SRS9+) parasite populations in these cell types at days 3, 5, and 7 post infection. Our results suggest that primary murine myocytes support brady-brady replication, while Caco-2 cells, a human gut epithelial cell line, do not. This data highlights the paramount influence of the host cell on the developmental pathway of the parasite. Our study leads the way to future experiments that aim to uncover the process that determines whether brady-brady replication occurs, a possible therapeutic target of chronic T. gondii infection.

18. Accelerating MalDA's anti-malarial drug discovery pipeline using an orthogonal DNA replication system in S. cerevisiae

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In 2022, approximately 249 million malaria cases were reported globally, with 608,000 deaths. Plasmodium falciparum and Plasmodium vivax are the predominant species causing malaria. The emergence of antimalarial drug resistance poses a significant threat to global efforts aimed at reducing the malaria burden. This highlights the need for new antimalarial drugs having novel mechanisms of action and underscores the importance of gaining a deeper insight into the mechanisms driving resistance. While conducting evolutionary studies directly in P. falciparum is time-consuming, Saccharomyces cerevisiae (yeast) offers a faster alternative due to its well-characterized genetics and a shorter replication time. In this study, we aim to leverage the OrthoRep system, an orthogonal replication system in yeast, to explore resistance-conferring mutations in P. falciparum drug targets. Specifically, the P. falciparum prolyl-tRNA synthetase (ProRS) gene was evolved against the antimalarial compound NCP-26, a known ProRS inhibitor. Using an error-prone DNA polymerase in OrthoRep, mutations were introduced at higher rates, driving rapid evolution of resistance. Preliminary results demonstrated that the evolved yeast strain exhibited resistance to NCP-26, with mutations arising in proximity to the drug-binding site, similar to those previously observed in P. falciparum. Additionally, Oxford Nanopore Sequencing revealed novel mutations that might be driving resistance towards NCP-26. This high-throughput system offers a promising tool for studying the mutational landscape of P. falciparum drug targets, accelerating the discovery of new antimalarial therapies. The insights gained from this yeast model can be further validated in P. falciparum, providing a streamlined approach to antimalarial drug development.

19. Characterization and functional analysis of Toxoplasma TBC domain Golgi proteins identifies TBC1 as a RAB7 GAP

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Toxoplasma gondii is an obligate intracellular parasite that infects nearly one-third of the human population, making it one of the most common parasites in the world. Its intracellular lifecycle is mediated by a series of unique organelles that play roles in motility, invasion, host-cell manipulation and parasite replication. Many of the proteins that target these organelles use the secretory pathway, but precisely how organellar sorting is achieved remains enigmatic. We previously characterized all of T. gondii's Tre2–Bub2–Cdc16 (TBC)-domain containing proteins, which are involved in vesicle fusion and intracellular trafficking between the ER-Golgi-Plasma Membrane. The 18 TBC-domain containing proteins in T. gondii localize to discrete regions of the secretory pathway, with four localizing specifically to the Golgi apparatus: an organelle essential for proper secretory traffic and cell survival. Knockouts of these four Golgi TBC proteins demonstrate that TBC1 is important to parasite's survival and lytic ability. Using mutagenesis, we show that TBC1 relies on a 'dual finger' active site within the TBC-domain. We additionally show by yeast 2-hybrid analyses that TBC1 binds Rab7, indicating that the TBC1-Rab7 pair is important for vesicular trafficking in the parasite. Together these studies provide new insights into protein trafficking in T. gondii and identify putative targets for the design of novel therapeutics that can specifically target the parasite.

20. Investigating the role for Bromodomain Protein 3 in initiating transcription of procyclin genes during differentiation of African trypanosomes from the bloodstream to the procyclic form

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Trypanosoma brucei, the causative agent of Human African Trypanosomiasis (HAT) and animal trypanosomiases, cycles between a bloodstream form in mammals and a procyclic form in the insect vector. In mammals the parasite evades the host immune system by antigenically varying its Variant Surface Glycoprotein (VSG). In flies, the parasite expresses one invariant procyclin protein from a limited number of EP and GPEET genes. The mechanism by which transcription of EP is initiated during differentiation is not well understood. We previously showed that occupancy of the bromodomain protein Bdf3, which binds to acetylated histones at transcription start sites, is dynamic throughout differentiation from the bloodstream form to the procyclic form in the fly midgut. However, de novo appearance of Bdf3 at new promoters during differentiation is rare, and only occurs at the EP and GPEET regions of the genome. In order to investigate whether Bdf3 is sufficient for activating transcription at the EP locus, we are using a CRISPR activation system to tether Bdf3 to the EP locus in bloodstream parasites that should not normally transcribe this procyclin gene. Using a GFP reporter system, we used flow cytometry to quantify the amount of EP transcription in the presence of a tethered Bdf3-dCas9 fusion protein and compared it to transcription of EP while tethering Cas9 alone. Preliminary experiments indicate that transient transfection of guide RNAs do not yield significant differences in transcription of EP, and stable transfection of guide RNAs may be necessary for optimal dCas9-mediated transcriptional activation. We hope these experiments will help us learn more about the mechanism by which EP transcription is initiated. Understanding the mechanisms of gene regulation in this highly diverged eukaryote may lend insight into how gene regulatory processes evolved across diverse biological systems.

21. Polyunsaturated fatty acids as weapons against Toxoplasma infection

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Intracellular pathogens such as the human parasite Toxoplasma gondii scavenge lipids from their host. Although host cells harbor reservoirs of potentially toxic lipids—the polyunsaturated fatty acids (PUFAs) that are highly susceptible to oxidation and are known to drive membrane damage and cell death—it is unknown whether host cells rewire lipid metabolism to thwart pathogen fitness. We found that, during T. gondii infection, PUFAs are selectively diminished from host triglycerides and incorporated into diacyl-PUFA-phospholipids (PL-PUFA2), which are mediators of oxidative membrane damage. The addition of PUFAs such as arachidonic acid to infected cells inhibits parasite proliferation and compromises its membrane morphology. The antimicrobial effect of arachidonic acid is reversed by addition of Ferrostatin-1, an inhibitor of lipid peroxidation. Using a CRISPR screen to define host regulators of T. gondii growth we identified GPX4, an enzyme that reduces peroxidized lipids to protect membranes from oxidative damage, as a candidate promoter of T. gondii growth. Our data suggests that host cells weaponize PUFAs as a cell-intrinsic innate immune-type defense to impair T. gondii growth, but that the parasite exploits GPX4 function to circumvent this defense.

22. The metabolic outcome of interventions on parasite-induced myocarditis and progressive cardiac fibrosis in chronic Trypanosoma cruzi-infected mice

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A vector-borne, intracellular parasite, Trypanosoma cruzi, causes Chagas disease (CD). T. cruzi is responsible for an estimated 6-7 million infections worldwide with 10,000 deaths annually. The CD is associated with a long-term change in the metabolic environment of individuals even after being treated. It is proposed that combinatorial interventions stemming from antiparasitics, immunotherapy, and STAT3 inhibition will improve T. cruzi-induced cardiac metabolic perturbations and advance cardiac health in Chronic Chagasic Cardiomyopathy (CCC). Metabolite analysis was done by subjecting the heart and liver tissues of the various treated mice to Liquid Chromatography-Mass Spectrometry (LC-MS) analysis, analyzing both polar and less-polar metabolites. The data were processed by MZmine, QIIME2, Random Forest, and GNPS, to determine the metabolites at play during the infection and the treatment interventions, to underscore which combination will likely restore the distressed tissues involved in the infection. Among the various interventions, TTI-101 produced prominent heart tissue restoration results, compared to other interventions in the infected groups. In the infected plus TTI-101 G4 intervention, the heart tissue restoration is closer to the G1 naïve mice. Partial heart tissue restoration was observed in infected plus vaccine plus TTI-101 G8 which was marginally closer to G1. In the low BZN plus TTI-101 infected G6 members partial heart tissue restoration was observed. A similar outcome of partial tissue restoration was encountered in G11 whose intervention was GLA-SE after infection. This initial investigation shows the promise of different treatment combinations toward tissue restoration during CCC in the host.

23. Gene prediction using machine learning and epigenetic features in Plasmodium falciparum

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In the recent few years, several studies have utilized various RNA sequencing technologies and strategies to discover the landscape of transcription in Plasmodium falciparum. Short and long read sequencing technologies facilitate the genome annotation in P. falciparum. However, truncated sequencing reads, transcriptional noise, transcription fragments and the overlap with ncRNAs may complicate genome annotation. Several of histone post-translational modifications (PTMs) in Plasmodium are recognized as universal markers of active promoters or euchromatin, which could aid in identifying undiscovered genes and validating known ones. In this study, we have used a machine learning approach for the detection of genes in the AT-rich genome of the human malaria parasite, P. falciparum, using exclusively epigenetic data. Using classifiers trained on comprehensive epigenetic datasets, we identified a total of 231 putative novel genes. Among these, 88% showed transcriptional signals in RNA-Seq data, indicating that our methodology is highly effective in gene identification and may also be useful for predicting lncRNAs in P. falciparum. Furthermore, by comparing the epigenetic patterns around gene boundaries with predictions from two genome annotations (PlasmoDB v68 and PlasmoDB v48), our findings suggest that the latest annotation (PlasmoDB v68) may have inappropriately extended the 5' and 3' UTR regions of certain protein-coding genes. Collectively, our results demonstrate that local chromatin structure provides sufficient information for genome annotation. Gene predictions based on epigenetic data could thus complement and enhance current sequence-based methodologies.

24. The dynamic interplay of human cells and Entamoeba histolytica during trogocytosis (cell-nibbling)

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Entamoeba histolytica is a pathogenic amoeba and causative agent of amoebiasis in humans. Despite its impact on health, E. histolytica is dramatically understudied. The species name (histo-: tissue; lytic-: dissolving) derives from the ability of E. histolytica trophozoites ("amoebae") to damage tissues, but the mechanism is not clear. We showed that amoebae kill human cells by biting off and ingesting cell fragments, referred to as trogocytosis (trogo-: nibble). Trogocytosis occurs in many other eukaryotes and may be fundamental to eukaryotic biology. However, the mechanistic differences between trogocytosis and phagocytosis (ingestion of entire cells) are unclear, as are the signals that initiate each process. When amoebae are incubated with live human cells, they take bites, but when amoebae are incubated with dead human cells, they engulf entire cells. Additionally, amoebae perform less trogocytosis, and more phagocytosis, when red blood cells were hardened by treatment with elevating glutaraldehyde concentrations. This suggests that the physical properties of human cells, such as actomyosin stiffness, are critical for amoebic trogocytosis. To test the role of host cell actin dynamics, we are using CRISPR interference (CRISPRi) in human cells to knockdown genes that modulate the actin cytoskeleton. Seven CRISPRi knockdown mutants have been successfully generated, with significant knockdown assessed by RT-qPCR. We are currently performing live cell imaging and quantitative assays to assess amoebic ingestion of these cytoskeletal mutants. These studies will improve understanding of trogocytosis, a process that appears to underlie amoebiasis pathogenesis, and may apply broadly to eukaryotic trogocytosis in general.

25. Estrogen dependent T cell regulation during toxoplasma infection

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Toxoplasma gondii (T. gondii) is an obligate intracellular parasite with the capacity to invade any nucleated cell in a wide range of host species, including humans. During chronic infection, the parasite transitions to a dormant state, forming tissue cysts in the brain and establishing a lifelong infection, often manifesting nonspecific clinical symptoms. A functional immune system, particularly Th1-mediated response and IFN-γ production, is essential for parasite suppression. Many types of immune cells, such as lymphocytes, express both estrogen receptors alpha and beta. It's known that steroid hormones, such as estrogen, play a crucial role in regulating innate and adaptive immune responses, with differences observed between males and females. For over 20 years we have known that female mice are more susceptible to Toxoplasma infection in part due to a decrease or delay in IL-12 production. Since this work there has been a growing understanding of the crosstalk between the immune and endocrine systems leading to sex-dependent susceptibilities to infection and disease. Here we have built upon the original studies and determined the inflammatory profile of immune cells in the brain of Toxoplasma chronically infected female and male mice. Our study demonstrates female mice have a higher infiltrating CD3+ T cells in the brain compared to male mice. To determine the specific role of the female specific hormone, estrogen on the enhanced inflammation during infection, we measured inflammation in ovariectomized female. These mice compared to both sham and non-surgery females, displayed significantly higher concentrations of serum cytokines, including IFN-γ, and an increase in immune cell infiltration to the brain, specifically CD4+, and CD8+ T-cell populations. Our results indicate that estrogen suppresses cytokine levels and immune cell populations in the brain during T. gondii infection. Future, experiments will test the role of the estrogen receptor on T cells for the protective or pathological response to Toxoplasma in the brain.

26. 3D spatial metabolomics of parasitic infection

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Parasites depend on their hosts for nutrient sources. In turn, hosts adapt to parasitic infections by reshaping their metabolism to compensate for or combat parasite nutrient uptake, facilitate tissue repair, and engage immune responses. The McCall laboratory leverages spatial metabolomics ("chemical cartography") to understand this chemical crosstalk, applying this technique to multiple parasitic diseases, including leishmaniasis, Chagas disease and toxoplasmosis. In the case of Chagas disease, symptoms include cardiac apical aneurysms, enlargement of the oesophagus and of the colon (megaoesophagus and megacolon). By systematically mapping infection-induced metabolic perturbations in the heart and gastrointestinal tract, we demonstrated that infection is associated with persistent alterations in overall metabolism at sites of Chagas disease tropism, even after parasite load is decreased by the immune system. Strikingly, these metabolically-perturbed sites are also distinct from the tissue sites of highest parasite load. In contrast, metabolism at sites that are not associated with disease tropism, such as the small intestine, goes back to normal once parasite levels decrease. Metabolic alterations are not restored by antiparasitic treatment, whereas immunomodulatory interventions improved metabolism. Metabolic families altered by infection include purines, acylcarnitines and phosphocholines. Overall, our results demonstrate a new, metabolomics-based method to define host-parasite interactions and intervene for parasitic disease treatment.

27. The role of microtubule polyglutamylation in the lytic cycle of Toxoplasma gondii

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Toxoplasma gondii is an obligate, intracellular parasite that causes a life-threatening disease in immunocompromised individuals and in the developing fetus due to congenital infection. Parasite morphology is mediated by a specialized microtubule-based cytoskeletal structure that is vital for facilitating host cell invasion. However, the mechanisms by which the cytoskeletal microtubules are regulated remain poorly understood. Two levels of regulation involve microtubule-associated proteins (MAPs) and post-translational modifications (PTMs) on the α- and β-tubulin heterodimers. In this study, we identified and localized all seven tubulin-tyrosine ligase like (TTLL) proteins in T. gondii using CRISPR/Cas9-mediated epitope tagging, that are predicted to play roles in tubulin modification, including polyglutamylation. We then used a tagging and knockout approach to show that the early daughter localizing TTLL-11A (TgGT1_244500) is responsible for microtubule polyglutamylation and is critical for the parasite's lytic cycle. Although Δttll-11a parasites do not display gross morphological defects, we show that the defect occurs at the step of invasion. We also utilized ultrastructure expansion microscopy (U-ExM) to better visualize the structure of the parasitic microtubules and cytoskeletal polyglutamylation. Together, these studies provide new insight into PTMs of microtubules in T. gondii and identify putative targets for the design of novel therapeutics that can specifically target the parasite.

28. Investigating NCR247's impact on helminth fitness using novel methods

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Helminth infections impact millions worldwide, and although antihelminthics are used to manage these infections, the rising resistance to these treatments indicates the need for novel therapeutic interventions. Our study investigates a recently identified potential therapeutic, NCR247, a heme-sequestering peptide that may deprive blood-feeding parasites, such as hookworms, of a molecule necessary for their development and reproduction. To measure its efficacy as a therapeutic established and innovative techniques such as ATP assays, morphological measurements, pigmentation via blood-feeding, and cuticle permeability tests against known anthelmintics, like Quinidine and Pyrantel Pamoate, to evaluate both mortality and extent of physical damage done to helminths. While our findings indicate that NCR247 does not effectively reduce helminth fitness, we successfully validated these novel methods using established antihelminthic treatments, highlighting these methods' potential and reliability for future studies on drug efficacy against helminths.

29. Optimizing immunity against toxoplasmosis by using exosomes released from Toxoplasma gondii and infected host immune cells

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Toxoplasma gondii is an apicomplexan parasite that infects a third of the human population through contaminated water or food. Acute infection is characterized by fast-replicating tachyzoites that differentiate into dormant bradyzoites. There are no viable vaccines for humans, and therapeutic strategies can lead to damaging side effects. Developing an ideal vaccine remains a significant challenge due to the complexities of the T. gondii life cycle. Previous studies have demonstrated exosomes released from tachyzoites lead to partial immunity in mice. Exosomes are extracellular vesicles (EVs) that carry essential cargo that play a role in cellular communication. Although previous studies have shown partial immunity, we aim to investigate if secretion from tachyzoites, bradyzoites, and T. gondii-infected immune cells could lead to long-lasting protection. To test this, we isolated EVs from both type II T. gondii life stages and infected immune cells (RAW-264.7). We then confirmed their presence by analysis of the vesicle surface marker CD63 and the life stage-specific proteins (SAG-1 and CST-1) using Western blot and confocal microscopy. To confirm exosome extraction, a nanoparticle tracking analysis will be conducted to measure particle size. After extraction, uninfected immune cells (RAW-264.7) were co-incubated with extracted exosomes to measure cytokine levels using ELISA. Additionally, we will immunize C57BL/6 mice by injecting the exosomes intramuscularly prior to infection. After T. gondii infection, survival rates will be monitored in addition to analysis of antibodies and cytokine levels using ELISA. This study will expand knowledge of the therapeutic potential for T. gondii-secreted exosomes in immunity against toxoplasmosis.

30. The Role of sex and the peripheral cannabinoid receptor on CB1R and CB2R mRNA expression in the brain and spleen of mice

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Cannabinoid receptors (CBRs) are an essential part of the endocannabinoid system (ECS) which is naturally found in most animals (vertebrates and invertebrates) but not insects. The ECS is involved in many physiological processes such as appetite, sleep, pain, memory, bone formation and immune responses. The best studied CBRs are the central cannabinoid receptor (CB1R), mainly found in the nervous system, and the peripheral cannabinoid receptor (CB2R), mainly found on immune cells. CBRs are activated by cannabinoids, either naturally produced by the body known as endocannabinoids, phytocannabinoids produced by plants or synthetic cannabinoids. CB1R expression is known to be altered by sex hormones, much less is known on how sex hormones affect CB2R expression. It is also known that CB2R expression is increased in CB1R knockout mice, but how CB2R deficiency affects CB1R expression is unknown. To determine the role of sex and CB2R deficiency on CB1R and CB2R mRNA expression, brains and spleens were obtained from male and female CB2R+/+ and CB2R-/- mice. CB1R and CB2R mRNA expression was determined by RT-PCR. Preliminary findings show that CB1R expression was present in brain and spleen but was about 200 times higher in the brain than in the spleen. Sex and CB2R deficiency did not seem to play a role in CB1R expression in the brain. CB2R mRNA expression was undetected in the brain. In the CB2R+/+ spleen, while we detected CB2R mRNA, CB1R mRNA expression was undetected. Sex did not seem to play a role in CB2R expression in the spleen.

31. Silencing regulatory transcription factors in N. caninum facilitates sexual cycle differentiation and provides insights into the mechanisms of merogony

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Neospora caninum, an obligate intracellular parasite infecting dogs and cattle, poses major health and economic challenges in the livestock industry. While N. caninum is closely related to T. gondii, it differs substantially in host relationships and disease impact: N. caninum affects cattle with canids as definitive hosts, while T. gondii impacts humans, sheep, and goats, using fields as definitive hosts. Importantly, humans are not hosts for N. caninum and the mechanism of this host restriction remains enigmatic. To understand N. caninum's lifecycle, we examine AP2 transcription factors, which regulate growth and stress responses in plants and are key to lifecycle transitions in apicomplexans. In T. gondii, AP2 factors such as AP2XII-1 and AP2XI-2 regulate the transition from tachyzoite to merozoite stages by gene silencing. Though present in N. caninum, AP2 roles remain largely unexplored in N. caninum. In this study, we endogenously tag AP2XII-1 and AP2XI-2 and conditionally deplete them using an auxin-inducible degron (AID) system. Depletion results in major morphological changes characteristic of the transition to the sexual cycle and reveals replication modes that resemble endopolygeny and schizogony. To confirm merozoite differentiation, we tagged the merozoite specific protein GRA80 and found it is robustly expressed upon AP2XII-1 and AP2XI-2 knockdown. We are currently using GRA80 immunoprecipitations and GRA80-TurboID strain to identify additional novel merozoite-specific GRAs. The development of a genetic control of differentiation enables us to explore how sexual development differs from T. gondii and may provide new insight into its unique sexual cycle and more limited host range.

32. NLRP3 inflammasome assembly and activation are mediated by endosomal trafficking-dependent pathway during Toxoplasma gondii infection of human immune cells

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The NLRP3 inflammasome mediates the processing of the pro-inflammatory cytokine IL-1β during Toxoplasma gondii infection of human peripheral blood monocytes. However, the cellular mechanisms that regulate the assembly of NLRP3 inflammasome components into a functional complex during T. gondii infection remain largely unknown. Utilizing super-resolution imaging in human monocytic cells (THP-1 cells expressing NLRP3-mNeonGreen), we visualized the trafficking dynamics of NLRP3 inflammasome components. NLRP3 was observed to co-localize with host microtubules and the microtubule-organizing center (MTOC) specifically in infected cells, with proximity ligation assays (PLA) revealing a close interaction between NLRP3 and α-tubulin. Real-time imaging further demonstrated that NLRP3 aggregates around the MTOC, displaying time-dependent movement from the cytoplasm towards the MTOC. Notably, NLRP3 particles exhibited bidirectional movement, with both retrograde transport toward the MTOC and anterograde movement toward the cell periphery. During its accumulation at the MTOC, NLRP3 was observed to move in a saltatory manner, similar to that seen with endosomal or lysosomal trafficking. Based on these observations, we treated cells with Vacuolin-1, an inhibitor of endosomal trafficking, and observed a reduction in NLRP3 activation in response to T. gondii infection in human monocytes. These findings offer new insights into the endosomal trafficking-dependent assembly of the NLRP3 inflammasome and its role in IL-1β processing during T. gondii infection of human immune cells.

33. Characterizing the relationship between Trichomonas vaginalis and Lactobacillus iners

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Lactobacillus iners is the most common bacteria found in the cervicovaginal microbiome. This bacterial community is most prone to shift towards a microbial community found in women with bacterial vaginosis. Infection with Trichomonas vaginalis is also associated with bacterial vaginosis. To our knowledge, nothing is known about T. vaginalis-L. iners interactions. We hypothesize that T. vaginalis exerts antibacterial effects on L. iners. We first optimized the conditions to maximally quantify L. iners colony formation on agar plates. Upon co-incubation with T. vaginalis, we found that co-incubation of both microbes at a ratio of 26-28 L. iners to 1 T. vaginalis, there was a statistically significant 95% reduction in L. iners colony formation in three biologically independent experiments. Interestingly, T. vaginalis viability was unaffected by the presence of L. iners. To visualize this antibacterial killing of L. iners by T. vaginalis, we utilized imaging flow cytometry to acquire single cell images of these interactions. We observed tight binding at 30 minutes, and T. vaginalis uptake of L. iners by 1 hour. Lastly, using transmission electron microscopy, we have found L. iners internalization by T. vaginalis. Delineating the factors that influence the mechanism of L. iners killing by T. vaginalis can potentially lead to the identification of novel therapeutic strategies to reduce the burden of T. vaginalis infections.

34. Using Toxoplasma strain-specific differences in encystment to identify genes essential for the bradyzoite stage

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Toxoplasma gondii is an intracellular parasite that chronically infects up to one-third of the world. Toxoplasma's chronic infection is mediated by switching from a fast-growing tachyzoite to a slow-growing bradyzoite that encysts in multiple cell types, including neurons. While two master regulators of stage conversion are known, the functions of the ~2000 downstream bradyzoite-specific genes are not. To address this gap, we analyzed RNA-seq data from primary murine neurons infected with a type II Toxoplasma strain (fast, efficient encystment) or a type III Toxoplasma strain (slow, inefficient encystment). We focused on genes upregulated in type II versus type III parasites and cross referenced these genes with "bradyzoite" genes identified through a publicly available dataset. This analysis identified 173 genes that we hypothesize are core genes linked to stage conversion and/or bradyzoite persistence. To test this hypothesis, we selected four genes that we confirmed were upregulated in an independent study of primary murine neurons infected with type II vs type III parasites. Three of the four are hypothetical proteins while the fourth was previously identified as being involved in brain colonization, though limited mechanistic work has been done on it. To define the role of these genes, we are currently generating individual knockouts and complemented strains in a type II strain. Once the knockout and complemented strains are generated, we will use in vitro assays and in vivo experiments to determine how each of these genes influences stage conversion and chronic infection.

35. Investigating the role for Bromodomain Protein 3 in initiating transcription of procyclin genes during differentiation of African trypanosomes from the bloodstream to the procyclic form

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Trypanosoma brucei, the causative agent of Human African Trypanosomiasis (HAT) and animal trypanosomiases, cycles between a bloodstream form in mammals and a procyclic form in the insect vector. In mammals the parasite evades the host immune system by antigenically varying its Variant Surface Glycoprotein (VSG). In flies, the parasite expresses one invariant procyclin protein from a limited number of EP and GPEET genes. The mechanism by which transcription of EP is initiated during differentiation is not well understood. We previously showed that occupancy of the bromodomain protein Bdf3 binds to acetylated histones at transcription start sites for the EP and GPEET regions of the genome. To investigate whether Bdf3 is sufficient for activating transcription at the EP locus, we are using a CRISPR activation system to tether Bdf3 to the EP locus using stable guide RNAs, and employing this system in bloodstream parasites that should not normally transcribe this procyclin gene. Using a GFP reporter system, we used flow cytometry to quantify the amount of EP transcription in the presence of a tethered Bdf3-Cas9 fusion protein. Preliminary experiments indicated an issue that the parent cell line that was not transfected with the stable guide RNAs was expressing a noticeable background level of GFP, and to address this, we used single cell cloning to isolate a parent cell clone that expressed a low level of GFP. Understanding the mechanisms of gene regulation in this highly diverged eukaryote may lend insight into how mechanisms of gene regulation evolved across diverse biological systems.

36. The role of reactive oxygen species (ROS) in controlling acute Toxoplasma gondii infection

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Toxoplasma gondii is an intracellular protozoan parasite that causes toxoplasmosis, a neglected infectious disease in the United States. Neutrophils are among the first innate immune cells to respond to this infection. These cells possess several antimicrobial mechanisms, including the production of reactive oxygen species (ROS). Although ROS has been observed in T. gondii-infected neutrophils, the molecular mechanisms associated with this response and its effectiveness in killing T. gondii have not been investigated. In neutrophils, ROS is primarily produced by NADPH oxidase 2 (NOX2). Here, we differentiated HL-60 cells into neutrophil-like cells (NLCs) and tested ROS production in response to two T. gondii strains. Type 2 (PA7) T. gondii induced high levels of ROS, unlike type 1 (RH), suggesting a potential mechanism of immune evasion from the latter. We also examined NOX2 expression and activation in infected neutrophils. Preliminary data showed constant gp91phox protein expression in cells infected with both T. gondii strains, however p40phox phosphorylation was observed only in type 2 infections, indicating that type 2 T. gondii-induced ROS is mediated by NOX2. We also tested the effectiveness of ROS in killing the type 2 strain through a plaque assay. The results suggested that, in the absence of opsonins, the effectiveness of ROS was relatively low. Future experiments will use human serum to investigate the role of opsonins in ROS production and T. gondii killing. We also aim to confirm NADPH oxidase activation by testing additional components of the enzyme.

37. Characterizing the role of Ctr2 in latent Toxoplasma gondii infection

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A vector-borne, intracellular parasite, Trypanosoma cruzi, causes Chagas disease (CD). T. cruzi is responsible for an estimated 6-7 million infections worldwide with 10,000 deaths annually. The CD is associated with a long-term change in the metabolic environment of individuals even after being treated. It is proposed that combinatorial interventions stemming from antiparasitics, immunotherapy, and STAT3 inhibition will improve T. cruzi-induced cardiac metabolic perturbations and advance cardiac health in Chronic Chagasic Cardiomyopathy (CCC). Metabolite analysis was done by subjecting the heart and liver tissues of the various treated mice to Liquid Chromatography-Mass Spectrometry (LC-MS) analysis, analyzing both polar and less-polar metabolites. The data were processed by MZmine, QIIME2, Random Forest, and GNPS, to determine the metabolites at play during the infection and the treatment interventions, to underscore which combination will likely restore the distressed tissues involved in the infection. Among the various interventions, TTI-101 produced prominent heart tissue restoration results, compared to other interventions in the infected groups. In the infected plus TTI-101 G4 intervention, the heart tissue restoration is closer to the G1 naïve mice. Partial heart tissue restoration was observed in infected plus vaccine plus TTI-101 G8 which was marginally closer to G1. In the low BZN plus TTI-101 infected G6 members partial heart tissue restoration was observed. A similar outcome of partial tissue restoration was encountered in G11 whose intervention was GLA-SE after infection. This initial investigation shows the promise of different treatment combinations toward tissue restoration during CCC in the host.

38. Visual Processing in Mice During Acute Toxoplasma gondii Infection

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Toxoplasma gondii is a food-borne pathogen that establishes lifelong infection in the central nervous system (CNS) of warm blooded hosts, including the brain and neural retina. The mammalian visual system begins with phototransduction in retinal cells and continues along the visual pathway to the cortex, where visually responsive neurons are activated. Behavioral impairments have been observed in T. gondii-infected mice, but it remains unclear whether sensory processing deficits contribute to these impairments. This study investigates whether T. gondii infection alters sensory processing in mice. Using intrinsic signal optical imaging (ISOI), we measured the strength of cortical response to visual stimuli in mice infected intraperitoneally with 200 type II T. gondii parasites. At 14 days post-infection, infected mice exhibited significantly reduced cortical response amplitudes compared to PBS-injected controls. This reduction in cortical responsiveness was associated with a decreased number of cFos+/NeuN+ neurons, indicating fewer neurons responded to the visual stimuli. Moreover, a larger proportion of cFos+ cells in the visual cortex were not NeuN+, suggesting an influx of non-neuronal cells, potentially linked to immune response. Future studies will focus on characterizing these non-neuronal cFos+ cells to better understand their role in infection-induced sensory deficits. Additionally, longitudinal studies will assess whether these sensory impairments persist or resolve during chronic infection. These findings may provide insights into the long-term consequences of T. gondii infection on CNS function and potential therapeutic targets.

39. Composition and in situ structure of the Methanospirillum hungatei cell envelope and surface layer

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Archaea share genomic similarities with Eukarya and cellular architectural similarities with Bacteria, though archaeal and bacterial surface layers (S-layers) exhibit key differences. Using cellular cryogenic electron tomography, we visualized the S-layer lattice surrounding Methanospirillum hungatei, a methanogenic archaeon. Though more compact than known structures, M. hungatei's S-layer is a flexible hexagonal lattice of dome-shaped tiles, uniformly spaced from both the overlying cell sheath and the underlying cell membrane. Subtomogram averaging resolved the S-layer hexamer tile at 6.4 Å resolution. By fitting an AlphaFold model into hexamer tiles of in flat and curved conformations, we uncover intra- and inter-tile interactions that contribute to the S-layer's cylindrical and flexible architecture, along with a spacer extension for cell membrane attachment. M. hungatei cell's end plug structure, likely composed of S-layer isoforms, further highlights the uniqueness of this archaeal cell. These structural features offer advantages for methane release and reflect divergent evolutionary adaptations to environmental pressures during early microbial emergence.

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