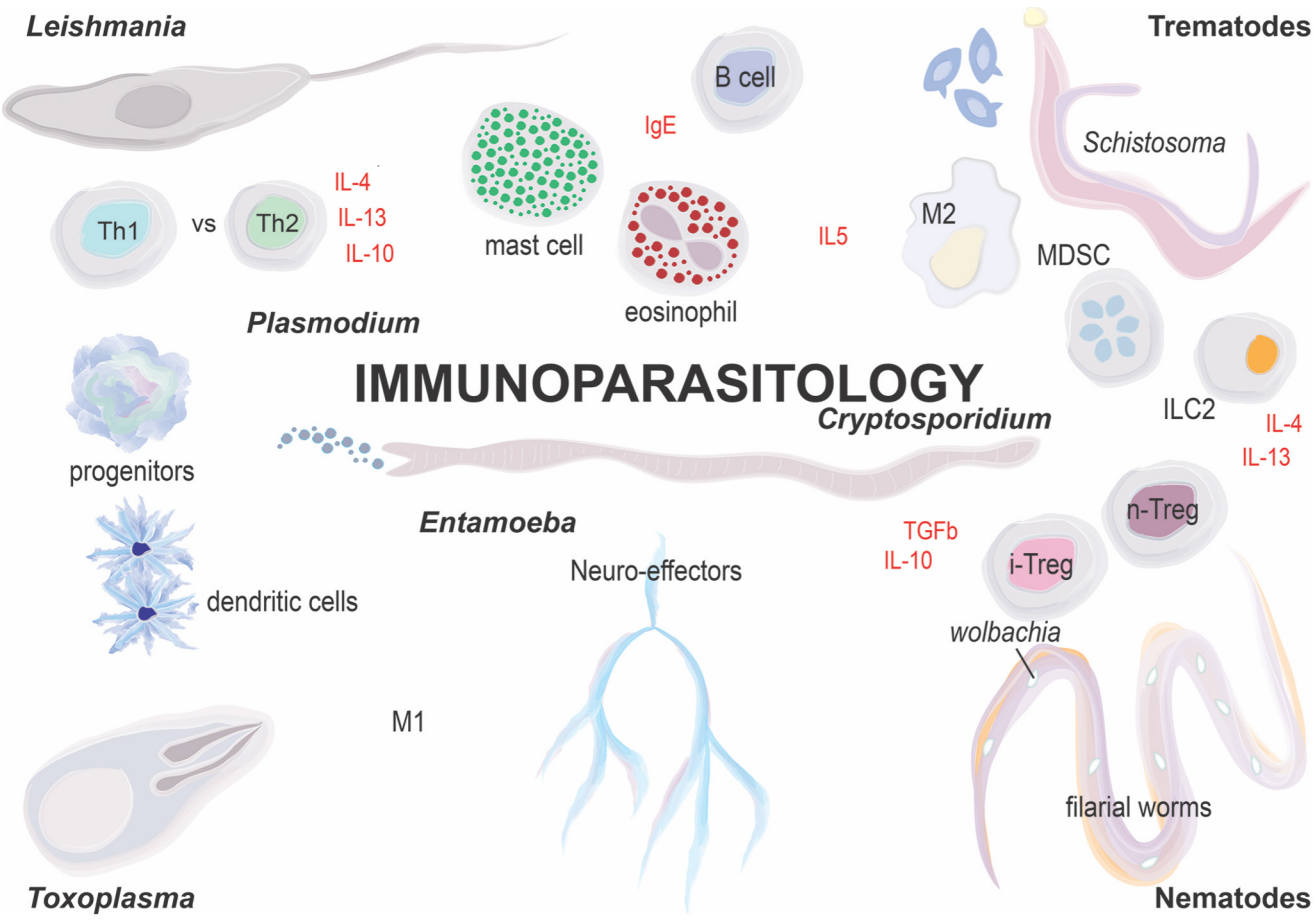

WHIP 2022

25th ANNUAL WOODS HOLE IMMUNOPARASITOLOGY MEETING

Marine Biological Laboratory

April 10 – 13, 2022

SCIENTIFIC PROGRAM

Sunday, April 10

Arrival and Registration (Swope Lobby)

4:45 PM – 5:00 PM **Opening remarks** (Clapp Auditorium)
Elia Tait Wojno, Jude Uzonna

5:00 PM – 6:00 PM **Keynote: The Biology of Intestinal Parasitism by *Cryptosporidium***
Boris Striepen, PhD (University of Pennsylvania)

6:10 PM Dinner (Swope)

8:00 PM Social Hour (Swope Lower Terrace Tent - Heated)

Monday, April 11

7:00 AM – 8:15 AM Breakfast (Swope)

8:45 AM – 10:30 AM **Session 1. Co-infection, Neuroimmunology, and Host-Pathogen Interactions**
(Clapp Auditorium)
Chairs: Tajie Harris and Nathan Peters (Aloe Stanbery, Zoom moderator)
(Jensen and Reese judging)

8.45am Plasmodium infection elevates risk of severe secondary bacterial disease by altering the immunological landscape of the lung
Jenna Reed, Douglas Cornwall, Nate Jacobs, Brian Evavold, and Tracey Lamb (University of Utah)

9.00am Intestinal helminths regulate systemic herpesvirus reactivation from tissue resident macrophages
Christina Zarek, Jaime Coronado, Phillip Dryden, and Tiffany A. Reese (University of Texas)

9.15am Meningeal lymphatic drainage promotes T cell responses against Toxoplasma gondii but is dispensable for parasite control in the brain
Michael A. Kovacs, Maureen N. Cowan, Katherine M. Still, Samantha J. Batista, Isaac Babcock, Lydia Sibley, Ish Sethi, and Tajie H. Harris (University of Virginia)

9.30am Understanding the effects of glutamate on mGluR+ CD8 T cells recruited to the T. gondii infected brain
WHIP 2022 Travel Award Winner
Edward A. Vizcarra, Tyler Landrith , and Emma H. Wilson (University of California, Riverside)

9.45am Monocytes maintain central nervous system homeostasis following helminth-induced inflammation
WHIP 2022 Travel Award Winner
Jianya Peng, Chandler B. Sy, John J. Ponessa, Alexander D. Lemenze, Christina M. Hernandez, Juan M. Inclan-Rico, Arman Sawhney, Hannah G. Federman, Krupa Chavan, Vanessa Espinosa, Sergei V. Kotenko, Amariliz Rivera, and Mark C. Siracusa (Rutgers-The State University of New Jersey)

10.00am Sugar modifications to the GPI regulate *Toxoplasma gondii* virulence
WHIP 2022 Travel Award Winner
Julia A. Alvarez, Jasmine Posada, Juan C. Sanchez-Arcila, Scott P. Souza, Elizabet Gas-Pascual, Christopher M. West, and Kirk D.C. Jensen (University of California, Merced)

10.15am The role of interferon-gamma in resistance to Cryptosporidium infection
WHIP 2022 Travel Award Winner
Ryan D. Pardy, Katelyn A. Walzer, Boris Striepen, and Christopher A. Hunter (University of Pennsylvania)

10:30 AM – 10:55 AM Coffee Break (Loeb Quad)

11:00 AM – 12:00 PM **Keynote: Understanding the Role of Helminth Antigens in Reprogramming Mammalian Immunity**
Keke Fairfax, PhD (University of Utah)

12:00 PM – 12:55 PM Lunch (Swope)

12:15 PM – 12:55 PM **Career Development Roundtables** (Swope)

1:00 PM – 2:30 PM **Session 2. Adaptive Immunity and Host Defense**
(Virtual, on Zoom in Clapp Auditorium)
Fernanda Novais and Chris Hunter (Oyebola Oyesola, Zoom moderator)
(Gazzinelli-Guimaraes and Wilson judging)

1.00pm *Plasmodium falciparum* coinfection Improves IgE and IgG3 response against Hookworm antigens
Samuel Asamoah Sakyi, Michael D. Wilson, Bright Adu, Stephen Opoku, Antwi Brewoo, Amma Larbi, Emmanuel Kyei Baafour, Samuel Kofi Tchum, Roland Osei Saahene, Wilfred Aniagyei, Christian Sewor, David Courtin, Michael Cappello, Ben Gyan, and Benjamin Amoani (Kwame Nkrumah University of Science and Technology)

1:15pm Skeleton binding protein-1-mediated parasite sequestration inhibits spontaneous resolution of malaria-associated acute respiratory distress syndrome
Hendrik Possemiers, Thao-Thy Pham, Marion Coens, Emilie Pollenus, Sofie Knoops, Sam Noppen, Leen Vandermosten, Sigrid D'haese, Luna Dillemans, Fran Prenen, Priyanka Koshy, Dominique Schols, Blandine Franke-Fayard, and Philippe E. Van den Steen (KU Leuven)

1.30pm The maintenance of Leishmania-specific CD4+ memory T cells requires the continuous presence of their cognate antigen
Zhirong Mou, Roma Zayats, Thomas Murooka, and Jude E. Uzonna (University of Manitoba)

1.45pm Whole-genome and ISG targeted sgRNA screen identifies novel restrictors of Toxoplasma infection in human cells
Sumit Kumar Matta and David L. Sibley (Washington University)

2.00pm Defining The Role Of Phosphatidylinositol 3-Kinase delta (Pi3kd) Pathway In B Cell Regulatory Function During Trypanosomal congolense Infection
Folayemi Olayinka-Adefemi, Chukwunonso Onyilagha, Nipun Jayachandran, Sen Hou, Ping Jia, Jude E. Uzonna, and Aaron Marshall (University of Manitoba)

2.15pm Exploring the role of the gut microbiota in tissue repair during intestinal helminth infection
Garrie Peng, Gabriel Russell, Susan Westfall, Cynthia Faubert, Siegfried Hapfelmeier, Irah King (McGill University)

2:30 PM – 2:55 PM Coffee Break (Loeb Quad)

3:00 PM – 4:45 PM **Session 3. Genetic, Metabolic and Environmental Cues in Immune Responses**
(Lillie)
Chairs: Kirk Jensen and Tiffany Reese (Oyebola Oyesola, Zoom moderator)
(Harris and Peters judging)

3.00pm Hypoxia promotes cytolytic activity of CD8 T cells and pathogenesis in cutaneous Leishmaniasis
WHIP 2022 Travel Award Winner
Erin Fowler and Fernanda O. Novais (The Ohio State University)

3.15pm Elucidating the Immunological Underpinnings of Asymptomatic Malaria
WHIP 2022 Travel Award Winner
Douglas H. Cornwall, Mellina T. Srey, Franklin M. Maloba, Birk K. Evavold, Adesola C. Olatunde, Anne Jensen, Tracey J. Lamb, and Brian D. Evavold (University of Utah)

3:30pm Investigating the contributions of hematopoietic progenitor cells to antihelminth immunity and host protection
Christina M. Hernandez, John J. Ponessa, Krupa Chavan, Jianya Peng, Hannah G. Federman, and Mark C. Siracusa (Rutgers-The State University of New Jersey)

3.45pm The Collaborative Cross reveals a single locus required for protective immunity against highly virulent Toxoplasma gondii strains
WHIP 2022 Travel Award Winner
Juan C. Sanchez-Arcila, Arlon Wizzard, Jennifer Eggleston, Darian Galvez, Scott P. Souza, and Kirk D.C. Jensen (University of California, Merced)

4.00pm Effects of environmental change on the immune system in Rhesus monkeys (*Macaca mulatta*)
Kasalina Kiwanuka, Jason M. Brenchley, and P'ng Loke (National Institute of Allergy and Infectious Diseases, NIH)

4.15pm Single cell RNA sequencing exploration of the effect of genetics and environment on immune composition and responsiveness in rewilded mice
WHIP 2022 Travel Award Winner
Nina G. Howard, Oyebola O. Oyesola, Alexander E. Downie, Ramya Smithaveni Barre, Ying-Han Chen, Kasalina Kiwanuka, Kimberly Zaldana, Soo Ching Lee, Joseph C. Devlin, Octavio Paloma Mondragon, Christin Herrmann, Sergei Koralov, Ken Cadwell, Andrea L. Graham, and P'ng Loke (National Institute of Allergy and Infectious Diseases, NIH)

4.30pm The skin microbiome enhances transcriptional inflammatory signatures and delays clinical resolution in cutaneous leishmaniasis
WHIP 2022 Travel Award Winner
Camila Farias Amorim, Victoria Lovins, Fernanda O. Novais, Jordan Harris, Lucas P. Carvalho, Edgar M Carvalho, Daniel P. Beiting, Elizabeth Grice, and Phillip Scott (University of Pennsylvania)

4:45 PM – 5:00 PM Stretch Break

5:00 PM **Poster Lightning Talks for In-person Attendees 1 slide/2 minutes**
(Clapp Auditorium)

5:30 PM **Poster Lightning Talks for Virtual Attendees**
(Virtual, on Zoom in Clapp Auditorium)

6:00 PM Dinner (Swope)

8:00 PM **In-person Poster Session & Social Hour**
(Meigs and Swope Lower Terrace Tent - Heated)

Tuesday, April 12

7:00 AM – 8:15 AM Breakfast (Swope)

8:45 AM – 10:30 AM **Session 4. Immunoparasitology: from Inflammation to Regulation**
(Virtual, on Zoom in Clapp Auditorium)
Chairs: Pedro Gazzinelli-Guimaraes and Emma Wilson (Mike Kovacs, Zoom moderator)
(Novais and Hunter judging)

8.45am Endogenous glucocorticoids promote survival in murine malaria by balancing inflammation and metabolism
Leen Vandermosten, Pauline Dagneau de Richecour, Fran Prenen, Sofie Knoops, Emilie Pollenus, Christopher Cawthorne, Sabine Vettorazzi, Christophe M. Deroose, Uwe Himmelreich, Jan Tuckermann and Philippe Van den Steen (KU Leuven)

9.00am Helminthic dehydrogenase drives PGE2 and IL-10 production in monocytes to potentiate Treg induction
Ulrich F. Prodjinotho, Vitka Gres, Fiona Henkel, Matthew Lacorcia, Ramona Dandl, Martin Haslbeck, Veronika Schmidt, Andrea S. Winkler, Chummy Sikasunge, Per-Johan Jakobsson, Philipp Henneke, Julia Esser-von Bieren, and Clarissa Prazeres da Costa (Technical University of Munich–TUM)

9.15am Homologues of the Heligmosomoides polygyrus IL-33 modulator, HpARI, displaying differing immuno-modulating properties
Florent Colomb, Adefunke Ogunkanbi, and Henry J McSorley (University of Dundee)

9.30am Small intestine immune interplay between host and gut microbiota modulates systemic adaptive immunity against Plasmodium yoelii
Rafael B. Polidoro, Olivia J. Bednarski, Morgan L. Waide, and Nathan W. Schmidt (Indiana University)

9.45am Kupffer cells death and changes in granuloma composition and function in experimental visceral leishmaniasis
Gabriela Pessenda, Tiago Rodrigues Ferreira, Andrea Paun, Eduardo Amaral, Sang Hun Lee, Sundar Ganesan, Olena Kamenyeva, Juraj Kabat, and David L. Sacks (National Institute of Allergy and Infectious Diseases, NIH)

10:00am RBP/J regulates tissue specific adaptation of group 2 innate lymphoid cells
Kyle Burrows, Louis Ngai, Edward Chen, Pailin Chiaranunt, Siu Ling Tai, Juan Carlos Zúñiga-Pflücker, and Arthur Mortha (University of Toronto)

10.15am Chronic brain neutrophils protect against Toxoplasma gondii infection
Kristina V. Bergersen, Bill Kavaathas, Byron D. Ford, and Emma H. Wilson (University of California, Riverside)

10:30 AM – 10:55 AM Coffee Break (Loeb Quad)

11:00 AM – 12:00 PM **Keynote: Monocyte-derived APC and signal 4 for T cell activation**
Tania Watts, PhD (University of Toronto)

12:00 PM – 12:55 PM Lunch (Swope)

1:00 PM – 2:30 PM **Session 5. Cellular and Molecular Innate Immune Responses** (Lillie)
Chairs: Michael Hsieh and Tracey Lamb (Ryan Pardy, Zoom moderator)
(Konradt and Scott judging)

1.00pm GSDMD deficiency drives immunopathology in cutaneous leishmaniasis
Christina K. Go, James C. Grayczyk, Igor Brodsky, and Phillip Scott (University of Pennsylvania)

1.15pm Understanding the mechanisms of immunity against percutaneous infection by a skin-penetrating Helminth
E. Evonne Jean and De'Broski R. Herbert (University of Pennsylvania)

1.30pm An unexpected role for cDC1s in Th1 responses to *Cryptosporidium*
Ian S. Cohn, Bethan Wallbank, Breanne Haskins, Ryan Pardy, Keenan O'Dea, Jennifer Dumaine, Jodi Gullicksrud, Boris Striepen, Christopher A. Hunter (University of Pennsylvania)

1.45pm Myeloid-derived IL-33 regulates keratinization and cutaneous IL-17 responses that prevent Schistosoma mansoni entry
Juan M. Inclan-Rico, Christopher F. Pastore, Li-Yin Hung, and De'Broski R. Herbert (University of Pennsylvania)

2.00pm The inflammatory role of caspase-8 during T. gondii infection of human monocytes
WHIP 2022 Travel Award Winner
Stephanie Matsuno, William Pandori, Tiffany Kao, Sharmila Mallya, Sarah Batarseh, and Melissa Lodoen (University of California, Irvine)

2.15pm IL-11 regulates mucosal immunity in pulmonary helminth infection
Jonah Kupritz, Pablo Bara-Garcia, Oyebola O. Oyesola, Fabricio Oliveira, Thomas B. Nutman, and Pedro H. Gazzinelli-Guimaraes (National Institute of Allergy and Infectious Disease, NIH)

2:30 PM – 2:55 PM Coffee Break (Loeb Quad)

3:00 PM – 4:30 PM **Session 6. T and B Cell Immunity** (Clapp Auditorium)
Christoph Konradt and Phil Scott (Ryan Pardy, Zoom moderator)
(Hsieh and Lamb judging)

3.00pm Helminth infection as a treatment for vitiligo in mouse model
Hanchen Li, Laura Lajoie, Donna Catalano, Xueli Fan, Rus Florentina, Gary R. Ostroff, Raffi V. Aroian, and John E. Harris (University of Massachusetts Chan Medical School)

3.15pm CD8+ T cells provide protection during Cryptosporidium Infection
Breanne Haskins, Jodi Gullicksrud, Jennifer Dumaine, Amandine Guerin, Bethan Wallbank, Ian Cohn, Keenan O'Dea, Ryan Pardy, Lindsey Shallberg, Emma Hunter, Jessica Byerly, Eleanor Smith, Briana Mcleod, Boris Striepen, and Christopher A. Hunter (University of Pennsylvania)

3.30pm Cellular dynamics of immune evasion during Leishmania major infection
WHIP 2022 Travel Award Winner
Romaniya Zayats, Zhirong Mou, Atta Yazdanpanah, Wan Hon Koh, Paul Lopez, Jude E. Uzonna, and Thomas Murooka (University of Manitoba)

3.45pm Sex-specific alterations in B cell development in a Maternal Schistosomiasis Model
Lisa C. Gibbs, Juan M. Oviedo, and Keke C. Fairfax (University of Utah)

4.00pm Th2 cells integrate into the tuft-ILC2 circuit to provide protective immunity to helminth infection
WHIP 2022 Travel Award Winner
Alison G. Stanbery, Lily Webeck, Jack McGinty, Jakob von Moltke (University of Washington)

4.15pm Crosstalk between microbiota and adaptive immunity determines susceptibility to amebic colitis
Md Jashim Uddin and William A. Petri Jr (University of Virginia)

4:30 PM **WHIP at 25 years and closing remarks**

6:00 PM Dinner (Swope Lower Terrace Tent - Heated)

7:15 PM **PI chat about the Future of WHIP** (Clapp Auditorium)

8:00 PM **In-person Poster Session & Social** (Meigs and Swope Lower Terrace Tent - Heated)

Thank you to our sponsors:

- The American Association of Immunologists
- National Institute for Allergy and Infectious Diseases
- Burroughs Wellcome Fund
- American Society for Hygiene and Tropical Medicine
- University of Pennsylvania School of Veterinary Medicine
- International Cytokine and Interferon Society

KEYNOTE SPEAKERS

The Biology of Intestinal Parasitism by *Cryptosporidium*

Boris Striepen, PhD (University of Pennsylvania)

Boris grew up in Ruhrort where the German rivers Rhine and Ruhr meet, an industrial area then dominated by coal and steel. He studied biology at the universities of Bonn and Marburg and conducted undergrad research on liver flukes in Bonn, and Nagana in Bobo Dioulasso, Burkina Faso. Boris earned a PhD summa cum laude for work on parasite biochemistry with Ralph Schwarz, was a postdoc with David Roos studying parasite cell biology, prior to starting his laboratory at the Center for Tropical & Emerging Global Diseases at the University of Georgia in 2000, where he last was a Distinguished Research Professor. In 2017 he joined the faculty of the University of Pennsylvania. Boris studies the cell and molecular biology of apicomplexan parasites. His current research focus is the parasite Cryptosporidium, a leading global cause of diarrhea and mortality in young children. His lab pioneered molecular genetics for this important infection and leads a range of interdisciplinary efforts to understand fundamental parasite biology and to advance translation towards drugs and vaccines. Boris is also engaged in education and training. He taught undergraduate and graduate classes, directed NIH training grant programs in parasitology, and served as faculty and director of the Biology of Parasitism summer research course at the Marine Biology Laboratories in Woods Hole, MA. Boris is married to a social worker with remarkable patience for scientists and has three children, two are scientists – all are awesome.

Understanding the Role of Helminth Antigens in Reprogramming Mammalian Immunity

Keke Fairfax (University of Utah)

Keke Fairfax received her PhD from Yale in Microbial Pathogenesis in 2009. Her dissertation work focused on identifying novel fatty acid binding proteins in the human hookworm Ancylostoma ceylanicum. She completed her post-doctoral training in Schistosoma mansoni immuno-parasitology with Edward Pearce and Gwendalyn Randolph in 2014. Dr. Fairfax began her independent laboratory at Purdue University in 2014 and moved to the University of Utah in 2018. The Fairfax laboratory at the University of Utah broadly focuses on using the helminth parasite Schistosoma mansoni as a tool to understand both, the relative contributions of schistosome antigen vs host IL-4 in inducing host immuno-modulation, and the complex interplay between lymphoid and stromal cells necessary to develop an optimal T and B cell memory response. Under this umbrella we currently have three main projects: 1) Understanding the immunological implications of maternal infections; 2) Dissecting the role of IL-4 in shaping the cellular environment of peripheral lymph nodes during homeostasis and antigenic challenge; 3) Delineating the mechanistic role of antigen driven immunological progenitor re-programing in helminth-induced protection from metabolic diseases.

Monocyte-derived APC and signal 4 for T cell activation

Tania Watts, PhD (University of Toronto)

Dr. Tania Watts received her Ph.D. in Biochemistry at the University of Alberta, followed by post-doctoral studies in Chemistry at Stanford University, where she developed an interest in Immunology. Dr. Watts joined the University of Toronto as an Assistant Professor in Immunology in 1986 and rose through the ranks to Full professor in 1997. Dr. Watts has had a long-term interest in immunity to viruses, focusing on how T lymphocytes respond and are regulated during acute and chronic infections, including responses to SARS-CoV-2 infection or vaccination. Dr. Watts' research focus for many years has been on regulation of T cell responses by TNFR superfamily members such as 4-1BB and GITR. We study TNFR signaling in T cells as well as cancers of the immune system. Dr. Watts is a former President of the Canadian Society for Immunology, Director of the Faculty of Medicine Flow Cytometry facility at the University of Toronto and was recently named a Distinguished fellow of the American Association of Immunologists, class of 2022. Her graduate mentorship was recognized by the 2019 JJ Berry-Smith award at the University of Toronto. Dr. Watts' research is funded by the Canadian Institutes for Health Research and by the Canadian Cancer Society.

ABSTRACTS

Session 1. Co-infection, Neuroimmunology, and Host-Pathogen Interactions

Plasmodium infection elevates risk of severe secondary bacterial disease by altering the immunological landscape of the lung

Jenna Reed (University of Utah)

Lower respiratory infections cause an estimated two million sepsis deaths yearly, with >50% attributed to the opportunistic pathogen Streptococcus pneumoniae. Sub-Saharan Africa, the site of most severe Streptococcus burden, is also ravaged by the malaria-causing Plasmodium parasite family, which afflicted 241 million people and killed 627,000 in 2020. Compared to healthy children, those with malaria have an elevated risk to severe bacterial infections, but the underlying mechanism(s) remain elusive. To fill this knowledge gap, we infected C57BL/6J mice with P. berghei NK65-NY either 7, 14, or 21 days prior to inoculation with S. pneumoniae 6304. Coinfected mice exhibited a 40-60% fatality rate, with death beginning 1-4 days post-bacterial infection. In contrast, singly infected groups survived the duration of the experiment. Coinfected mice exhibited equivalent parasitemia but significantly higher bacterial burden in the lung compared to mono-infections. Neutrophil recruitment and ROS production was altered in coinfected mice, which we predict is due to the formerly described effects of the Plasmodium metabolic product hemozoin. As such, we hypothesize that neutrophil control of S. pneumoniae is impaired. Since malaria has been shown to increase blood brain barrier permeability, we used Evans blue dye to test for a similar effect at the alveolar-capillary interface. Lung weight significantly increased as early as 6 days post-infection, while permeability trended towards a slight increase, peaking at day 9. Thus, we hypothesize that Plasmodium infection increases pathogenicity of Streptococcus infection due to a combination of impaired bacterial control and increased vascular leak in the lungs.

Intestinal helminths regulate systemic herpesvirus reactivation from tissue resident macrophages

Tiffany Reese (University of Texas)

Many helminth infections are strictly confined to the intestines, but they have the ability to regulate systemic immune responses. Despite the recognized power of helminth infections to change immune responses to secondary pathogens and alter development of immune-mediated diseases, we still do not understand the mechanisms for helminth-induced immune suppression or disease promotion. We are investigating the influence of parasite infections on resident tissue macrophages and co-infection with viruses. We found that infection of mice with the intestinal helminth, Heligmosomoides polygyrus, expanded and altered the function of the resident population of macrophages in the peritoneal cavity called large peritoneal macrophages (LPM). When mice with intestinal parasite infection were co-infected with murine gammaherpesvirus-68, a herpesvirus which established latent viral infection, the virus had increased infection and reactivation in the LPMs. This increase in virus reactivation did not required canonical IL-4/IL-13 signaling via Stat6. However, it did require dietary vitamin A and retinoic acid. We propose a model whereby intestinal parasite infection expands LPMs in a vitamin A dependent manner, which alters the establishment of herpesvirus latency and reactivation in LPMs.

Meningeal lymphatic drainage promotes T cell responses against Toxoplasma gondii but is dispensable for parasite control in the brain

Michael Kovacs (University of Virginia)

Toxoplasma gondii is an intracellular protozoan parasite that causes chronic brain infection in a wide range of mammalian hosts. Animal studies have demonstrated that continuous T cell recruitment to the brain is necessary for parasite control. However, it has remained unclear how T cells outside the central nervous system sense and respond to brain-derived microbial antigen. Here, we test the hypothesis that the newly described meningeal lymphatic system promotes T cell immunity during T. gondii brain infection. We find that chronic brain infection is associated with significant expansion of parasite-specific T cells in the cerebrospinal fluid (CSF)-draining deep cervical lymph nodes. T cell activation at this site is correlated with parasite burden in the brain and peaks during the late stages of chronic infection. Flow cytometric analysis of CSF reveals a population of activated dendritic cells that is not present in the CSF of naïve mice. Mature dendritic cells are also mobilized in the meninges, specifically in areas where CSF protein can be sampled. Disrupting meningeal lymphatic drainage by ligating the collecting vessels leads to impaired T cell responses in the deep cervical lymph nodes. Surprisingly, in spite of reduced T cell activation and IFN-gamma production at this site, T cell responses in the brain remain intact, likely due to ongoing antigenic stimulation in lymph nodes that drain non-central nervous system tissue. Overall, we provide evidence that meningeal lymphatic drainage supports robust parasite-specific T cell responses in the deep cervical lymph nodes. Nonetheless, we find that drainage of central nervous system material to this site is dispensable for host-protective T cell responses in the brain.

Understanding the effects of glutamate on mGluR+ CD8 T cells recruited to the T. gondii infected brain

Edward Vizcarra (UC Riverside)

Toxoplasma gondii (T. gondii) is one of the most effective transmissible pathogens in the world, infecting approximately two billion people. Encystment of the parasite in neurons in the brain results in a lifelong chronic infection. Within the brain, a pro-inflammatory response is essential to prevent disease from parasite reactivation. Infection in the immunocompromised leads to lethal Toxoplasmic encephalitis while in the immunocompetent, there is persistent low-grade inflammation which is devoid of clinical symptoms. This suggests that there is a tightly regulated inflammatory response to T. gondii in the brain. T cells are required to control parasite replication through secretion of effector molecules such as perforin and IFNɣ. However, the regulation of these cells in this critically important tissue is poorly understood. During chronic infection there is an increase in extracellular (EC) glutamate that is normally tightly controlled in the brain. High EC glutamate is not specific to T. gondii infection and can occur during multiple pathologies in the CNS, but may be an important environmental signal to tissue specific immune cells. We hypothesize that this glutamate-rich environment plays a role in T cell function and regulation.

Here we demonstrate that CD8 T cells from the T. gondii -infected brain express the G-protein coupled metabotropic glutamate receptors (mGluR's) mGluR1 and mGlur5. This expression is enriched in T cells recruited to the brain compared to secondary lymphoid-derived cells. Furthermore, single cell RNA-sequencing and flow cytometry data suggests that mGluR expression on CD8 T cells confers a memory phenotype. We further hypothesize that T cells recruited to the brain are regulated by glutamate through mGluR modulation. Using activators and inhibitors of these receptors we will test glutamate dependent signaling mechanisms that are implicated in T cell function and regulation in response to T. gondii in the chronically infected brain.

Monocytes maintain central nervous system homeostasis following helminth-induced inflammation

Jianya Peng (Rutgers)

Neuroimmune interactions are crucial for regulating immunity and inflammation. Recent studies have revealed that the central nervous system (CNS) senses peripheral inflammation and responds by releasing molecules that limit immune cell activation, thereby promoting tolerance and tissue integrity. However, the extent to which this is a bi-directional process, and whether peripheral immune cells also promote tolerance mechanisms in the CNS remains poorly defined. Here we report that helminth-induced type 2 inflammation promotes monocyte responses in the brain that are required to inhibit excessive microglial activation and host death. Mechanistically, infection-induced monocytes express YM1 that is sufficient to inhibit TNF production from activated microglia. Importantly, neuroprotective monocytes persist in the brain and infected mice are protected from subsequent LPS-induced neuroinflammation months after infection-induced inflammation has resolved. These studies demonstrate that infiltrating monocytes promote CNS homeostasis in response to inflammation in the periphery and demonstrate that a peripheral infection can alter the immunologic landscape of the host brain.

Sugar modifications to the GPI regulate *Toxoplasma* gondii virulence

Julia Alvarez (University of California, Merced)

The development of an effective vaccine against parasitic infections like Toxoplasma gondii requires more understanding about the battle between host and pathogen. We are exploring the role the GPI anchor plays in T. gondii virulence and immune evasion. The glycosylphosphatidylinositol (GPI) anchor is a highly conserved glycolipid that anchors proteins to the external membrane of T. gondii and is found in all eukaryotes. While the core structure of the GPI is conserved, species differ in sugar modifications made to the core structure, called "side chains". GPI-lipids (GPILs) of T. gondii are known to be recognized by innate pattern recognition receptors TLR-2 and -4 and are robustly targeted by IgM antibodies after infection with T. gondii. However, the role of the glycosyl side chain of the GPI is unknown. We have successfully characterized and knocked out the glycosyltransferase responsible for GalNAc addition to the mannose backbone of the GPI in T. gondii. Parasites lacking this enzyme have complete loss of both GalNAc and GalNAc+Glc GPI glycoforms, which allowed us to explore how modifications to the GPI impact parasite virulence, a first for any pathogen. These mutant parasites, including type III "nonvirulent" strains, have increased virulence and parasite burden, and decreased proinflammatory cytokine responses and IgM recognition of GIPL, demonstrating that the GPI sidechain modulates parasite virulence. We are currently exploring mechanisms by which the glycosylated side chain of the GPI is required for proper immune detection and resistance mechanisms to T. gondii, but suspect evasion of innate immune detection underpins several of these phenotypes associated with the GPI mutant strains.

The role of interferon-gamma in resistance to Cryptosporidium infection

Ryan Pardy (University of Pennsylvania)

Cryptosporidium species are intracellular parasites that infect epithelial cells (EC) in the small intestine and can cause severe long-term effects for young or immunocompromised patients. Interferon-gamma (IFN-gamma) plays a crucial role in protective immunity, as IFN-gamma KO mice are highly susceptible and often succumb to infection, however, how IFN-gamma-mediated resistance is poorly understood. Our laboratory has shown that during Cryptosporidium infection, IL-12 and enterocyte-derived IL-18 stimulates innate lymphoid cell (ILC) production of IFN-gamma. Further, mice with an enterocyte-specific deletion of STAT1 (STAT1-IEC KO), which is a part of the IFN-gamma signaling cascade, and to a lesser extent mice deficient for the IFN-gamma-induced effectors Irgm1 and Irgm3 show increased susceptibility to Cryptosporidium. Treatment of WT or IFN-gamma KO mice with recombinant IFN-gamma prior to challenge does not prevent establishment of infection but dampens parasite burden. Further, while Irgm1/m3 are important for parasite control and clearance, the protective effect of exogenous IFN-gamma was not dependent on these proteins. Using a reporter mouse for IFN signaling, we show that IFN-gamma treatment signals directly to intestinal EC, and the protective effect of treatment was abrogated in STAT1-IEC KO mice. Finally, single-cell RNA sequencing of intestinal EC identified IFN-gamma-dependent gene signatures and potential candidate genes for further investigation. Together, these studies provide new insights into the role of IFN-gamma in the immune response to Cryptosporidium.

Session 2. Aaptive Immunity and Host Defense

Plasmodium falciparum Coinfection Improves IgE and IgG3 response against Hookworm Antigens

Benjamin Amoani (University of Cape Coast)

Background: Plasmodium falciparum and Hookworm infections are prevalent in Sub-Saharan Africa and common cause of iron deficiency anaemia and protein malnutrition in Children. Immune responses from these parasites interact and their interactions could have implications on vaccine development and efficacy. The current goal on hookworm eradication lies on vaccination. We evaluated the effect of Plasmodium falciparum coinfection and albendazole treatment on naturally acquired antibody profile against hookworm L3 stage larvae antigen. Methods: In a longitudinal study, a total of 139 participants consisting of 59 individuals infected with Necator americanus (Na) only, 63 participants with Necator americanus and P. falciparum (Na-Pf) coinfection and 36 non-endemic controls were recruited. The study was conducted in the Kintampo North Municipality of Ghana. Blood and stool samples were taken for laboratory various analysis. Serum samples were obtained prior to hookworm treatment and three weeks after treatment. Results: The Malaria-hookworm (Na-Pf) co-infected individuals had significantly higher levels of IgE [$\beta = 0.30$, 95% CI = (0.12, 0.48), $p = 0.023$] and IgG3 [$\beta = 0.15$, 95% CI = (0.02, 052), $p = 0.004$] compared to those infected with hookworm only (Na). The Na groups had significantly higher levels of IgG3 [$\beta = 0.39$, 95% CI = (0.14-0.62), $p = 0.002$] compared to the control group. Furthermore, the Na-Pf co-infected participants had significantly higher levels of IgE [$\beta = 0.35$, 95% CI = (0.70-0.39), $p = 0.002$] and IgG3 [$\beta = 0.54$, 95% CI = (0.22-0.76), $p = 0.002$] to hookworm L3 stage antigens compared to the negative endemic control (NEC) group. Conclusion: P. falciparum improves IgE and IgG response against hookworm L3 stage larvae. Treatment with single dose of albendazole led to reduction in naturally acquired immune response against hookworm infection. Thus, P. falciparum infection could have a potential boosting effect on hookworm vaccine efficacy.

Skeleton binding protein-1-mediated parasite sequestration inhibits spontaneous resolution of malaria-associated acute respiratory distress syndrome

Hendrik Possemiers (KU Leuven)

Malaria is a hazardous disease caused by Plasmodium parasites and often results in lethal complications. Parasite sequestration in the microvasculature is often observed, but its role in malaria pathogenesis and complications is still incompletely understood. We used skeleton binding protein-1 (SBP-1) KO P. berghei NK65 parasites to study the role of sequestration in experimental malaria-associated acute respiratory distress syndrome (MA-ARDS). The sequestration-deficiency of these SBP-1 KO parasites was confirmed with bioluminescence imaging and by RT-qPCR, and resulted in a lower parasitemia and parasite load. The SBP-1 KO parasites induced similar lung pathology in the early stage of experimental MA-ARDS compared to wildtype (WT) parasites. Strikingly, the lung pathology resolved subsequently in more than 60% of the SBP-1 KO infected mice, resulting in prolonged survival despite the continuous presence of the parasite. This spontaneous disease resolution was associated with decreased inflammatory cytokine expression and lower expression of cytotoxic markers in pathogenic CD8+ T cells in the lungs. Moreover, the infected mice developed also malaria-associated acute kidney injury (MAKI) pathology with glomerular and tubular damage, proteinuria and renal inflammatory cytokine expression. SBP-1 KO did not affect this MAKI pathology. These data suggest that SBP-1-mediated parasite sequestration and subsequent high parasite load are not essential for the development of experimental MA-ARDS and MAKI but inhibit the resolution of the lung pathology.

The maintenance of Leishmania-specific CD4+ memory T cells requires the continuous presence of their cognate antigen

Zhirong Mou (University of Manitoba)

Memory T cells play essential role in immunity against viruses, bacteria and protozoan parasites. Although it has been shown that memory CD8+ T cells are maintained as a stable pool for extended time periods in the absence of their cognate antigen, whether memory CD4+ T cells are sustained in the absence of antigen is controversial. Leishmaniasis is caused by the protozoan parasite belonging to the genus Leishmania. Most individuals who recover from cutaneous leishmaniasis acquire a lifelong immunity to reinfection. This infection-induced immunity is believed to be dependent upon the presence of persistent parasites although empirical evidence supporting this is lacking. We found the mice infected with dihydrofoliate reductase thymidylate synthase (dhfr-ts) deficient L. major, which are incapable of surviving due to inability to salvage thymidine, lost protection against wild-type L. major challenge in 24 weeks, which corresponds to loss of Leishmania (PEPCK)-specific CD4+ T cells. To test the fate of Leishmania-specific memory CD4+ T cells in absence of antigen, we generated Leishmania PEPCK-specific CD4+ TCR transgenic mice (PEG). Using this unique tool, we found that both in vitro and in vivo generated memory PEG cells disappeared over time in both MHC II KO or WT mice and this was associated with loss of protection following challenge infections. Similarly, following adoptive transfer of PEG cells and infection with dhfr-ts L. major or immunization with PEPCK peptide, PEG cells underwent cell expansion and contraction but completely disappeared in about 300 days. PEG cells also did not persist in mice infected with either dhfr-ts or PEPCK deficient L. major, but did persist in mice infected with WT parasites. The disappearance of memory PEG cells in either dhfr-ts or PEPCK deficient L. major infected mice was associated with loss of protection after rechallenge infections with WT L. major. Taken together, these results show that the maintenance of antigen-specific memory CD4+ T requires the continuous presence of their cognate antigen.

Whole-genome and ISG targeted sgRNA screen identifies novel restrictors of Toxoplasma infection in human cells

Sumit K. Matta (Washington University)

Type II interferon (IFN-gamma) is a critical host mediator for innate control of many pathogens including Toxoplasma gondii that causes toxoplasmosis in humans. Human cells treated with IFN-gamma show cell-type specific restriction mechanisms including nutrient limitation, recruitment of autophagy adaptors, guanylate-binding proteins (GBPs) and host cell death. However, previous studies have failed to identify a common factor or pathway of parasite control in IFN-gamma treated human cells. We executed whole-genome and IFN-gamma induced ISG (Interferon Stimulated Genes) CRISPRCas9 sgRNA screens in A549 lung epithelial cells to identify factors responsible for IFN-gamma induced control of T. gondii growth. Surprisingly our screen failed to identify canonical ISGs normally thought to explain the antimicrobial effects of IFN-gamma. Instead, we identified NF2 and RNF213 as novel regulators of T. gondii growth. NF2 or merlin, is a member of the Ezrin/Radixin/Moesin (ERM) family of proteins. Our studies reveal that NF2 is necessary for maximal IFN-regulatory factor 1 (IRF1) transcriptional activity, which is necessary for upregulation of ISGs downstream of IFN signaling. In contrast, RNF213, a E3 ubiquitin ligase was involved in ubiquitinylating the parasitophorous vacuole surrounding the parasite, thus promoting recruitment of autophagy adaptors leading to growth restriction in different human cell types. CRISPR-cas9 generated knockout lines demonstrated that RNF213 is the primary ubiquitin ligase recognizing the parasitophorous vacuole in human cells and is required for IFN-gamma mediated growth restriction. This study highlights that CRISPR-Cas9 screens can be used to identify novel factors involved in control of intracellular survival and growth of eukaryotic pathogens like Toxoplasma.

Defining The Role of Phosphatidylinositol 3-Kinase delta (Pi3kd) Pathway in B Cell Regulatory Function During Trypanosomal congolense Infection.

Folayemi Olayinka-Adefemi (University of Manitoba)

Introduction:PI3Kd is important for B-cell responses to model antigens however, it's role in regulatory B-cell functions in the context of infectious immunity is broadly unknown.

Methods: Intraperitoneal (ip) Infection of PI3Kd-loss-of-function mutant mice (PI3Kd-LOF/GL) and PI3Kd-B-cell specific hypermutant mice (PI3Kd-GOF/B), with 1000 parasites of the B-cell targeting protozoan Trypanosoma congolense (TC13), Flow cytometry, ip treatment of C57BL/6 (WT-control) mice with a PI3Kd-inhibitor: Idelalisib, Cytokine analysis by Mesoscale ELISA, Griess Assay for Nitric Oxide (NO). Results: Mice with a genetic loss-of-PI3Kd (PI3Kd-LOF/GL) have a surprising ability to control parasitemia in early infection (7-9 days), despite impaired lymphocyte activation. Drug (Idelalisib) inhibition of PI3Kd from WT-mice similarly produced improved parasite control, consistent with findings in the genetic mutants. Peritoneal fluid and blood analysis for cytokines, as well as immunophenotyping of the peritoneum indicates significant elevations in IFN-gamma (favorable in early disease) and reduction in the suppressive cytokine, IL-10

during early infection. Our results suggest that IL-10 producing B1 cells (regulatory B cells) localized to the peritoneum are the key cells responsible for regulating early immune responses. These cells fail to develop in the PI3Kd-LOF/GL mutants while their IL-10 secretory functions are suppressed in WT-mice treatment with Idelalisib. Preliminary results indicate that mice with a B cell-specific hyperactivation of PI3Kd (PI3Kd-GOF/B), show a reciprocal effect of impaired early control of parasitemia associated with expanded regulatory B- cell populations. New results show that these regulatory B-cells are potentially carrying out their function via unique mechanisms like the adenosine pathway (CD39/CD73), distinct from IL-10. Interestingly, the assessment for Nitric oxide (indicative of favorable host responses from macrophages) show elevation of NO in the PI3Kd inhibited mice and a reciprocal decrease in NO in the PI3Kd hyperactivated mice. Conclusion:PI3Kd-signalling impacts a network of regulatory innate immune responses critical for prompt protection in early Trypanosomal infection.

Session 3. Genetic, Metabolic and Environmental Cues in Immune Responses

Hypoxia promotes cytolytic activity of CD8 T cells and pathogenesis in cutaneous Leishmaniasis

Erin A.L. Fowler (The Ohio State Univeristy)

Leishmaniasis is a disease caused by protozoan parasites of the genus Leishmania and the most common form of the disease is cutaneous leishmaniasis (CL). A fundamental question in CL is what regulates the development of severe disease, information that is critical to develop therapies to ameliorate disease. In a series of studies, we demonstrated CD8 T cell-dependent cytotoxicity as the main inducer of immunopathology in CL. This result was unexpected since IFN-ɣ production by CD8 T cells plays a protective role by promoting pathogen elimination. To resolve this paradox, we studied the CD8 T cells in different anatomic sites and found that the effector function of CD8 T cells in CL depends on their location: while CD8 T cells are cytotoxic (GzmB+) and produce little IFN-ɣ in leishmania lesions, CD8 T cells in the draining lymph nodes (dLN) have the opposite profile. Importantly, GzmBCD8 T cells from dLN quickly upregulate GzmB after injection into CL lesions. By transcriptional profiling, we found that CD8 T cells in lesions and not dLN have a hypoxic signature. In vivo, we observed that leishmania lesions are hypoxic using the Oxyphor G4 oxygen probe and pimonidazole staining. In vitro, we found that induction of hypoxia was sufficient to convert GzmB- into GzmB+ CD8 T cells. In vivo, blocking the dimerization of HIF-α, a master regulator of hypoxia, with acriflavine decreased lesion development in mice. Transcriptional profiling in patients showed that hypoxia is a signature of human CL and correlates with GZMB expression. Together, our results suggest that the hypoxic microenvironment of leishmania lesions alter the function of CD8 T cells and convert protective CD8 T cells into pathogenic cytotoxic T cells.

Elucidating the Immunological Underpinnings of Asymptomatic Malaria Utilizing a Novel Genetically Diverse Wild Mouse Model

Douglas H Cornwall (University of Utah)

With over 200 million clinical cases per year that lead to over 600,000 deaths malaria is still a significant global health problem. Up to 80% of individuals in some malaria-endemic areas can have asymptomatic carriage of Plasmodium infections (no overt symptoms) and this is thought to be a significant reservoir for malaria transmission. It is appreciated that the term asymptomatic is a misnomer, as many of these individuals display evidence of mild anemia and vascular activation and have a significantly higher risk for co-morbidities such as infection with typhoidal Salmonella. Currently the immunological mechanisms governing the asymptomatic carriage of Plasmodium are poorly understood and this is partly due to a lack of asmptomatic animal models that are immunologically intact. Here, we utilize a unique genetically diverse wild-derived mouse model to address the immunological underpinnings of asymptomatic malaria. This model allows us to disentangle the relative roles of the genetics and environment in the generation of immune responses to Plasmodium infections. Results show the wild-derived mice have varying levels of anemia associated with non-lethal Plasmodium yoelii XNL infection, with some mice exhibiting an asymptomatic phenotype with mild anemia independently of parasitemia. Anemia levels positively correlate with the production of TNF-α and IL-10, which is consistent with patterns seen in children in Cameroon. Furthermore, the CD4 T cells producing IL-10 and IFN-ɣ were also correlated with the severity of anemia, also a pattern seen in human malaria. Her we have applied GWAS analysis to elucidate previously unknown genes associated with the asymptomatic phenotype in these mice. These results demonstrate that host genetics play a key role in facilitating asymptomatic malaria and that the wild-derived mice can be used to model asymptomatic malaria allowing interrogation of the immune mechanisms leading to anemia.

Investigating the contributions of hematopoietic progenitor cells to antihelminth immunity and host protection.

Christina M. Hernandez (Rutgers-The State University of New Jersey)

Protective responses to helminth parasites are dependent on type 2 cytokine-mediated inflammation that is required for worm expulsion and the healing of damaged tissues. These events are critically supported by various cell populations including mast cells and erythrocytes. Our previous studies have recently identified a population of hematopoietic progenitor cells (HPCs) that possess dual mast cell and erythrocyte potential. These dual progenitors are defined by their expression of the metabolic enzyme carbonic anhydrase (Car)1 and traffic to inflamed tissues following a Trichinella spiralis infection. Importantly, Car1-expressing progenitors contribute to host protection by simultaneously supporting mast cell responses that promote type 2 inflammation and erythrocyte development that combats infection-induced anemia. Despite these advances, the relative contributions of Car1-expressing progenitors to the infection-induced increases in these cell types remain unknown. To address this, we have developed a novel Car1-Cre mouse to perform important fate-mapping studies. Here we demonstrate that our novel mouse model can be used to selectively label Trichinella-induced mast cells with a history of Car1-expression. Further, we demonstrate that Car1-Cre mice can also be employed to selectively delete Car1-expressing progenitors. This novel tool will allow us, for the first time, to evaluate the developmental origins of infection-induced mast cells. Further, this newly generated mouse model will also permit loss-of-function studies to test the contributions of a specific progenitor cell to antihelminth immunity and host protection.

The Collaborative Cross reveals a single locus required for protective immunity against highly virulent Toxoplasma gondii strainse immunity to Toxoplasma gondii

Juan Camilo Sanchez Arcila (University of California Merced)

We employed an unbiased forward genetics screen to determine new requirements for immunity to T. gondii against a highly virulent strain of T. gondii, the causative agent of human toxoplasmosis. To detect immunity QTLs generated by vaccination or natural infection with a low-virulent strain, we screened the available panel of 59 lines of the Collaborative Cross (CC), a highly diverse genetic panel of inbred mice captures ~90% of the genetic variation within the Mus musculus species. We challenged immunized mice with a highly virulent French Guyana strain, GUY-DOS, capable of evading immunological memory responses in some but not all founder CC lines. We found one highly significant Quantitative Trait Locus (QTL) in a small region on chr11 that correlated with survival to GUY-DOS secondary infections in vaccinated or naturally infected animals. The chr11 QTL accounts for 70% of the total phenotypic variance in the CC. The protective effects to GUY-DOS in the CC mice were related to PWK/PhJ and CAST/EiJ genetic backgrounds. The number of mutations and GO enrichment of genes in the chr11 QTL indicates that the most probable candidate in the region corresponds to Tcf7, a known regulator of CD4+ T follicular and CD8+ T central memory lymphocytes. A further evaluation revealed higher TCF-1 expression in bulk splenocytes, enhanced memory CD8+ T cell, humoral and Tfh responses following vaccination in CAST/EiJ compared to C57BL/6J. To test the specific role of chr11 from strains associated with protection during the QTL mapping, we applied the same infection model to consomic B6.PWD-chr11.1 mice and observed protection against the challenge with the highly-virulent strain VAND, suggesting a pivotal role of chr11 in mediating protection against high-virulent strains. Thus, candidates located in the chr11 QTL, with a potential contribution of Tcf7, may explain the immunological basis for enhanced vaccine efficacy against virulent challenge.

Effects of environmental change on the immune system in Rhesus monkeys (*Macaca mulatta*)

Kasalina Kiwanuka (NIAID/NIH)

Geographical areas with a higher prevalence of infectious disease and parasitic infection have a lower rate in atopic disease. Immigrants can develop atopic disease within a few years of migration to industrialized regions or within a generation to rates equivalent to native individuals, suggesting that the environment plays a role in training and shaping the immune system. Rhesus macaques used at the National Institutes of Health are captured on a free ranging Indian Origin Rhesus Outdoor Breeding Colony then transported to quarantine facilities where they are treated with anti-helminthic medications and quarantined before deployment into various studies. The movement of Rhesus macaques from the outdoor breeding colony to animal facilities mimics human immigration from a geographical areas of high infection prevalence to areas of low infection prevalence (de-wilding). Thus, this system affords the unique opportunity to study how change in environment affects the immune system. Analysis of whole blood pre and post quarantine revealed a change in the neutrophil:lymphocyte ratio, with a significant decrease in neutrophils and increase in lymphocytes post-quarantine. Stimulation assays with microbial antigens coupled with analysis of cytokine production indicated that there may be a bias away from a TH17 to a more TH1 like cytokine profile post-quarantine. Collectively these data suggest a significant impact of changes in the environment on the immune and hematological system of monkeys after “de-wilding”. This might be a useful model analogous to the migration of human populations to a more industrialized environment.

Single cell RNA sequencing exploration of the effect of genetics and environment on immune composition and responsiveness in rewilded mice

Nina Howard (National Institute of Allergy and Infectious Diseases, NIH)

Immune responses during helminth infection vary significantly among individuals, with some inducing optimal protective responses, while others launch responses that are too strong or too weak. Host genetic profile and environmental factors could influence an individual's immune response, but the relative contribution and interactions of these components remains largely unknown. Single cell sequencing offers a novel approach to address this question, as it allows us to distinguish groups of immune cell populations and to study heterogeneity between cells. Here, we use single cell RNA sequencing to assess the contribution and interaction of host genotype and environment to the immune cell landscape. This was accomplished by releasing genetic inbred strains of mice, 129-SL, PWK and C57/B6, to a rewilded environment and then infecting them with an intestinal helminth parasite, Trichuris muris. Sequencing was then performed on the mesenteric lymph nodes of mice, resulting in a dataset of 49,700 cells from 122 mice. Based on previous studies, we hypothesized immune cell composition would be driven by environment, while genotype would drive cytokine activity. However, when we clustered cells and identified cell populations, results indicated genotype has the largest effect on the composition of cells in the mesenteric lymph nodes of mice. We also found genotype has the biggest effect on the types of cytokines expressed among cells with cytokine activity. We did see environmental cell composition differences, with rewilded mice having a greater proportion of Follicular B cells and lab mice having more CD4 T cells. We also found rewilded mice have a significantly greater percentage of cells exhibiting cytokine activity compared to lab mice. This suggests environment does play a role in immune composition and cytokine activity, but contrary to our initial hypothesis, genotype has a greater effect than environment on both of these aspects of the immune cell landscape.

The skin microbiome enhances transcriptional inflammatory signatures and delays clinical resolution in cutaneous leishmaniasis

Camila Farias Amorim (University of Pennsylvania)

Cutaneous leishmaniasis (CL) caused by Leishmania braziliensis is associated with chronic lesions that are often difficult to drug treat. We previously found that treatment failure is associated with increased expression of cytolytic genes, including GZMB, GNLY and PRF1, as well as IL1B. Here we investigate how the skin microbiome influences host gene expression in lesions and treatment outcome. We carried out an integrative multi-omics study from 64 L. braziliensis patients including RNA-seq from lesion biopsies, 16 seq from skin swabs collection of bacterial isolates prior to treatment. We first assessed the total bacterial burden in lesions by qPCR of the 16S ribosomal subunit and found that patients with higher bacterial burdens exhibited delayed healing. To identify the bacteria, we performed 16S sequencing of lesion swabs and found that Staphylococcus was the most frequent dysbiosis observed in patients and was associated with delayed lesion resolution. Since 50% of the Staphylococcus isolates, we collected were S. aureus, and S. aureus can be associated with severe infections, we asked whether lesions with high levels of S. aureus might be associated with inflammatory gene expression. We generated an in-house Staphylococcus aureus pangenome from our clinical isolates and known public references to quantify S. aureus transcript abundances through dual RNA-seq mapping analysis. We found that lesions with increased S. aureus transcripts exhibited high expression of inflammatory-related genes, such as CXCL5/8, CCL3/4, IL1A, IFNG, as wells as genes we previously reported as biomarkers for treatment failure including PRF1, GNLY, GZMB and IL1B. Together, these results suggest that the skin microbiome influences immune responses in lesions of CL patients, affecting how patients respond to therapy with antimony leading to a delay in healing. These studies suggest that antibiotics or probiotic therapies given in conjunction with anti-parasitic drugs might augment healing.

Session 4. Immunoparasitology: from Inflammation to Regulation

Endogenous glucocorticoids promote survival in murine malaria by balancing inflammation and metabolism

Leen Vandermosten (KU Leuven)

During malaria, the hypothalamic-pituitary-adrenal axis is activated and glucocorticoid (GC) levels are increased. However, the role of endogenous GCs in malaria has been barely investigated despite its versatile effects on immune responses, vascular function, and metabolism. These processes are all crucial in malaria and often get disturbed in patients leading to, amongst others, hypoglycemia and lactic acidosis.

Infection of C57BL/6 mice with Plasmodium chabaudi AS (PcAS) leads to a considerable self-resolving peak of parasitemia, along with mild symptoms and liver inflammation. Our data shows that the spleen size and splenic glucose uptake drastically increase upon this infection. Glycogen is broken down to meet the increased glucose demand, but the mRNA expression of the gluconeogenic enzyme

glucose-6-phosphatase is strongly reduced.

We have proven that adrenal hormones protect against early death in several mouse models of malaria by preventing excessive systemic and brain inflammation and severe hypoglycemia. The contribution of other adrenal hormones besides GCs (e.g. aldosterone and/or catecholamines) could however not be ruled out. Because most effects of GCs are mediated by the glucocorticoid receptor (GR), we used tamoxifen-inducible global GR knockout mice. We here show that the absence of the GR in PcAS-infected mice impairs survival, which coincides with severe hypoglycemia, glycogen exhaustion, increased cerebral pro-inflammatory cytokine expression and more glucose uptake in spleen and liver.

Altogether, disruption of the HPA axis in murine malaria models clearly results in increased inflammation and lethal hypoglycemia, which is prevented by exogenous GC treatment. Limited data suggest that relative corticoid insufficiency might occur in some malaria patients. More in-depth studies into this relative corticoid insufficiency in malaria may hold promise for a better understanding and treatment of specific malaria complications such as severe hypoglycemia.

Helminthic dehydrogenase drives PGE2 and IL-10 production in monocytes to potentiate Treg induction

Fabien Ulrich Prodjinotho (Technical University of Munich)

Immunoregulation of inflammatory, infection-triggered processes in the brain constitutes a central mechanism to control devastating disease manifestations such as epilepsy. Observational studies implicate the viability of Taenia solium cysts as key factor determining severity of neurocysticercosis (NCC), the most common cause of epilepsy, especially in children, in Sub-Saharan Africa. Viable, in contrast to decaying, cysts mostly remain clinically silent by yet unknown mechanisms, potentially involving Tregs in controlling inflammation. Here, we show that the enzyme glutamate dehydrogenase from viable cysts instructs tolerogenic monocytes to release IL-10 and the lipid mediator PGE2. These act in concert, converting naive CD4+ T cells into brain homing CD127-CD25hiFoxP3+CTLA-4+CCR6+CCR7+ Tregs, through the G protein-coupled receptors EP2 and EP4 and IL-10 receptor. Moreover, while viable cyst products strongly upregulate IL-10 and PGE2 transcription in microglia, intravesicular fluid, released during cyst decay, induces proinflammatory non-phagocytic microglia and TGF-β as potential drivers of epilepsy. Inhibition of PGE2 synthesis and IL-10 signaling prevents Treg induction by viable cyst products. Harnessing PGE2-IL-10 axis and targeting TGF-ß signaling may offer an important therapeutic strategy in inflammatory epilepsy and NCC.

Homologues of the *Heligmosomoides polygyrus* IL-33 modulator, HpARI, displaying differing immuno-modulating properties

Florent Colomb (University of Dundee)

The IL-33 pathway is critical for ejection of many helminth parasites, including the rodent nematode Heligmosomoides polygyrus. H. polygyrus has evolved multiple mechanisms by which it can modulate the IL-33 pathway, including the secretion of HpARI, a protein which binds directly to IL-33 and to DNA, tethering the cytokine within necrotic cells.

Here, using in-house analysis of the H. polygyrus transcriptome and proteome, we identify and describe a family of proteins, HpARI1, HpARI2 and HpARI3, which although they share strong sequence similarity, show differing binding affinity for IL-33 and DNA. Importantly, those differing biochemical features appears to translate into variable activities against IL-33. While HpARI1 appears to have a minimal role in vivo, HpARI2 binds and blocks IL-33 responses, as described in our previous work. In contrast to HpARI2, HpARI3 appears to amplify (rather than suppress) IL-33 responses through stabilisation of the cytokine. HpARI3 has a lower affinity for IL-33 compared to HpARI2, which appears related to its IL-33 stabilisation. Intriguingly, HpARI3 also appears to entirely lack the capability to bind to DNA shown by HpARI2.

By testing mutants, truncations and chimeras of the HpARI family in in vivo and in vitro IL-33 assays, we are characterising how these homologues disparate activities are mediated. Furthermore, we are assessing the expression of each homologue across the H. polygyrus lifecycle, and their activity during infection. We currently hypothesise that H. polygyrus may suppress or amplify IL-33 in order to suppress type 2 immunity, or activate regulatory responses, at different stages of infection.

Small Intestine Immune interplay between host and gut microbiota modulates systemic adaptive immunity against Plasmodium yoelii

Rafael B. Polidoro (IUSM)

Our lab has previously demonstrated that gut microbiota composition can modulate the severity of malaria using mice from different vendors, regular (Tac) and hyperparasitemic mice (CR). We found gut microbiota dependent decreases in the stability of germinal center B cells and parasite-specific antibody titers between these two vendors. These findings were inversely correlated with parasite burdens of the mice. To further characterize the gut impact, we harvested the Peyer Patches (PP), MLN, ILN and spleens from the mice before and on various days during an infection. We found PP differences between Tac and CR at all timepoints, suggesting an active immune response at this site, with Tac mice maintaining lower T follicular helper (TFh) and higher Tregs and T follicular regs (TFr) in the PP before the infection, suggesting a healthy homeostasis. Differently, CR mice present active GC B and TFh cells in their PP prior to the infection, suggesting a dynamic homeostasis with immune activation in the small intestine. Flow cytometry of IgA+ fecal bacteria confirms that CR mice have higher basal IgA+ bound bacteria, and both vendors have a proportional increase of IgA bound bacteria from day 8-10. Gavage of purified IgA bound bacteria into Tac mice before an infection is sufficient to reproduce the hyperparasitemic phenotype. Chemical induction of intestinal damage on CR mice 10 days before the infection causes the non-lethal severe malaria model (Plasmodium yoelii) to become partially lethal, suggesting that the intestinal dysbiosis caused by malaria systemic infection further enhances the bacterial damage and impedes a proper systemic immune response against malaria. These results provide a mechanistic insight into the impact of the gut microbiota on the extra-gastrointestinal tract immune response and will be an important factor to consider in the development of optimal antimalarial treatments.

Kupffer cells death and changes in granuloma composition and function in experimental visceral leishmaniasis

Gabriela Pessenda (NIAID/NIH)

Visceral leishmaniasis (VL) is a tropical disease caused by protozoan parasites of the genus Leishmania (L. donovani/L. infantum). Human VL is transmitted by sand fly bites and is fatal in the absence of treatment. Macrophages in the liver, spleen, and bone-marrow are the principal target cells for infection. Kupffer cells (KCs) are the embryonic derived (emKCs), liver resident macrophages, and are characterized by Clec4f and Tim4 expression. In homeostasis, KCs maintain their numbers via self-proliferation, but in some inflammatory settings they can die and be replaced by monocyte-derived cells (moKCs). In the murine VL model, KCs are important for both initial parasite growth and granuloma formation, which is associated with the eventual protective response. KC death, their replacement by moKCs, and the functionality of emKCs vs moKCs has never been investigated in VL. In C57BL/6 mice infected with 3 million metacyclic L. infantum, at 42 days post-infection we found evidence of KC apoptosis and ferroptosis. Consistently, Clec4f and Tim4 expression in KCs was reduced, while monocyte markers such as Ly6C and CD11c were enhanced. As further evidence of their monocytic origin during chronic infection, KC frequency was lower in infected CCR2-/- mice when compared to infected WT mice, and in experiments involving congenic parabiotic mice, the KC population bore either congenic marker at 42 d.p.i. Interestingly, granuloma cores were composed mainly of Clec4f- KCs, suggesting that during chronic infection most granulomas are composed of moKCs. Selective KC depletion and replacement by moKCs prior to infection, using Clec4f-Cre-DTR mice, demonstrated that predominance of moKCs resulted in lower parasite loads, suggesting that moKCs are more effective in controlling infection. Further investigation on the mechanisms of KC death and how changes in granuloma composition affect parasites and/or tissue damage will provide additional insights into how KCs are able to resolve the liver infection.

RBP/J regulates tissue specific adaptation of group 2 innate lymphoid cells

Kyle Burrows (University of Toronto)

Recombination signal binding protein for immunoglobulin kappa J region (RBP/J) is a key effector molecule of Notch signaling that facilitates lineage specification of precursors towards the T cell fate and enforces GATA3 expression in Th2 cells. Group 2 innate lymphocytes (ILC2) also require GATA3 for their differentiation while early ILC precursor development has been proposed to rely on Notch signaling. However, the exact role for RBP/J in ILC2 differentiation and function has not been fully elucidated. Here we use an in vivo conditional RBP/J expression system to facilitate timed and inducible expression of RBP/J in hematopoietic cells to investigate the role of RBP/J in ILC2 development. Using a combination of bone marrow chimeras and adoptive cell transfers, we report that RBP/J-deficiency yields an anticipated block of T cell development but failed to disrupt ILC2 development across all innate lymphoid development stages. Instead, RBP/J-deficient ILC2s displayed an RBP/J-dependent alteration of ST2 expression in the gut but not the lung or adipose tissue. The modulated cytokine receptor expression led to hyperactivation of gut ILC2s and elevated production of IL-5 and IL-13, which resulted in an expansion of both goblet cells and tuft cells within the intestinal epithelium. Induced expression of RBP/J in RBP/J-deficient ILC2s, or neutralization of IL-33 reversed the IL-5 and IL-13-driven changes in the intestinal epithelium. Taken together, these results indicate that RBP/J dependent activation of Notch signaling in ILC2s constitutes a local gut-specific signal that drives tissue-adaptation of these innate lymphoid cells.

Chronic brain neutrophils protect against Toxoplasma gondii infection

Kristina Bergersen (University of California Riverside)

Infection with the protozoan parasite Toxoplasma gondii leads to the formation of lifelong cysts in neurons of the brain that can have devastating consequences in the immunocompromised. However, despite the establishment of a chronic inflammatory state and infection-induced neurological changes in the brain, most neurons show no overt signs of clinical pathology resulting in an asymptomatic infection in the immunocompetent. This suggests the work of neuroprotective mechanisms to prevent clinical manifestations of disease. However, such sources of neuroprotection during infection remain largely unknown. Previous work in other models of central nervous system (CNS) injury and infection demonstrates neuroprotection by CNS-resident and peripheral immune cells via the expression of neuroprotective molecules. Targeted gene expression analysis during chronic Toxoplasma infection has also previously demonstrated upregulation of neuroprotective genes and repair pathways.

We have identified a population of chronic neutrophils in the brain during Toxoplasma infection that expresses neuroprotective molecules including NRG1, ErbB4, and MSR1. Further phenotyping of this chronic neutrophil population via flow cytometry and singe-cell RNA sequencing reveals two distinct subsets of neutrophils that display functional heterogeneity. Furthermore, chronic depletion of neutrophils results in increased parasite burden and infection-induced vascular pathology. Lack of neutrophils during chronic infection deleteriously affects neuronal regeneration and repair indicating a direct neuroprotective role of these cells. Collectively, these results demonstrate a novel neutrophil population that may play a dynamic role in controlling chronic infection and inflammation in the CNS by balancing classical responses with pro-resolution functions.

Session 5. Cellular and Molecular Innate Immune Responses

GSDMD deficiency drives immunopathology in cutaneous leishmaniasis

Christina Go (University of Pennsylvania)

Cutaneous leishmaniasis is a neglected tropical disease that results in severe, chronic lesions that are frequently resistant to parasite-directed therapy. Studies in patients and murine models indicate that NLRP-3 dependent IL-1β release is associated with more severe disease and treatment failure. One mechanism leading to IL-1β release as well as pyroptosis is the formation of pores by gasdermin D (GSDMD). To determine if GSDMD might contribute to IL-1β release and subsequent increased disease, we infected GSDMD knockout mice and wild-type C57BL/6 (WT) mice with Leishmania major and followed the course of infection. Contrary to our expectations, GSDMD KO mice infected mice developed more severe lesions compared with WT mice, but without a change in parasite burden. Because inflammasome activation without pyroptosis has been reported to lead to prolonged IL-1β release from hyperactivated dendritic cells, we tested whether blocking IL-1β signaling would prevent severe pathology in GSDMD KO mice. However, GSDMD KO mice treated with anti-IL-1β developed comparable pathology to untreated GSDMD KO mice, indicating that IL-1β was not mediating the increased pathology. Therefore, we next compared the immune responses in WT and GSDMD KO mice. A significant increase was found in the production of IFNɣ, and our working hypothesis is that excess IFN-ɣ may contribute to disease severity. Why the absence of GSDMD leads to increased disease remains unclear, but previously it was found that TNFα and type I IFN are increased in GSDMD KO mice. Current studies are focused on defining the mechanism(s) beyond inflammasome signaling that lead to increased disease in GSDMD KO mice.

Understanding the Mechanisms of immunity against percutaneous infection by a Skin-Penetrating helminth

E. Evonne Jean (University of Pennsylvania)

Soil transmitted helminths (STH) cause chronic disease and significant morbidity in billions of people worldwide. Most STH species have an infectious third-stage larvae (iL3) that penetrate the skin of its host to establish parasitism. Strongylodies ratti is a rodent specific STH that mimics key features of pathogenesis caused by the human parasite S. stercoralis and used to understand Type 2 immunity. While considerable work has revealed the importance of hematopoietic cells in host resistance, the role of sensory neurons is less comprehensive in resolving and preventing larvae penetration and migration. Skin sensory neurons are known to serve key roles in immunity against cutaneous microbial and fungal pathogens through the induction of neutrophils and Th17 cells, but the role of neurons in immunity against skin-penetrating STH infections is currently unexplored. This gap is largely due to needle-based inoculation methods that bypass neurons innervating the skin. To address this issue, we developed an infection system wherein the mouse skin surface is transiently exposed to a saline bath of iL3, allowing natural penetration. Interestingly, data show that wild-type mice acquire resistance to skin penetration by iL3 following prior exposure and expression of neuropeptides increases. We asked what mechanisms lead to repulsion of cutaneous penetration by S. ratti iL3. Data shows that CD4 T Cells are indispensable for acquired resistance to penetration in a manner independent of STAT6,

an important transcription factor mediating many Type 2 immune responses. Future studies will determine the contributions of skin innervating neurons in augmenting host resistance through activation of CD4 T Cells, and/or neutrophils and Type 17 cytokines.

Myeloid-derived IL-33 regulates keratinization and cutaneous IL-17 responses that prevent Schistosoma mansoni entry.

Juan M. Inclan-Rico (University of Pennsylvania)

The skin is a structural and immunological barrier that prevents pathogen entry, but mechanisms of skin immunity against helminths, such as Schistosoma mansoni remain unclear. The alarmin cytokine IL-33 is widely considered a product of damaged structural cells that mediates immune cell function(s) through its receptor T1/ST2 expressed on myeloid and lymphoid cell populations. In contrast, this study addressed whether CD11c-expressing cells could serve as an important source of IL-33 for regulation of skin homeostasis and immunity against S. mansoni cercariae. Data show that IL-33 is expressed by several subsets of skin myeloid cells at baseline. Surprisingly, selective deficiency myeloid-derived IL-33 augmented IL-17/IL-23 and γδ T cell responses and increased keratinization with epidermal thickening at the steady-state. These basal changes were associated with IL-17/IL-23 mediated resistance to cercarial skin penetration relative to WT controls. To investigate potential mechanisms, single cell RNAseq was performed on sort-purified skin myeloid cells, revealing that selective deficiency of IL-33 resulted in expansion of several myeloid cell subsets. Further, these myeloid subsets exhibit an altered transcriptional profile with increased expression of cytokines such as IL-1β, suggesting that IL-33 may control pro-inflammatory cytokine secretion by myeloid cells. Indeed, IL-33-deficient bone marrow-derived macrophages or dendritic cells secrete elevated levels of cytokines compared to controls when stimulated with TLR ligands (LPS) or DAMPs (ATP). Collectively, these results highlight a previously unrecognized regulatory circuit by which IL-33 intrinsically limits cytokine secretion by cutaneous myeloid cells to prevent heightened γδ T cell activity and keratinocyte proliferation that can prevent the entry of helminths through the skin.

The inflammatory role of caspase-8 during T. gondii infection of human monocytes

Stephanei Matsuno (University of California Irvine)

Toxoplasma gondii is a food-borne obligate intracellular parasite that infects one-third of the global human population. Innate immune cells, such as monocytes, are among the first cells recruited to sites of infection and produce the potent proinflammatory cytokine IL-1β. We previously showed that T. gondii-infected primary human monocytes produce IL-1β through a Syk-PKC-δ-CARD9-MALT1-NF-κB signaling pathway, and IL-1β release requires the NLRP3 inflammasome and caspase-1 activity. To investigate a potential role of other caspases in IL-1β release, we conducted CRISPR/Cas-9 genome editing to knock out caspase-1, -4, -5, or -8 in THP-1 cells. Genetic ablation of caspase-1 or -8, but not caspase-4 or caspase-5, decreased IL-1β release during T. gondii infection. Furthermore, dual pharmacological inhibition of caspase-8 with IETD and RIPK1 with necrostatin-1 in primary human peripheral blood monocytes decreased IL-1β release without effecting cell viability or infection efficiency. Caspase-8 was not required for the production or cleavage of IL-1β but rather, caspase-8 inhibition led to the retention of mature IL-1β within the cells. In investigating caspase-8 processing, we found that infection with type II T. gondii, which induces IL-1β release, leads to cleavage of caspase-8 from full-length p57 protein to the p30 subunit. In contrast, type I infection, which does not induce IL-1β release, did not trigger caspase-8 cleavage. Our data suggest that during type II T. gondii infection of human monocytes, caspase-8 functions in a novel gasdermin D-independent mechanism controlling IL-1β release from viable cells. This study expands on the molecular mechanisms of IL-1β release from human immune cells and on the inflammatory role of caspase-8 in host defense.

IL-11 regulates mucosal immunity in pulmonary helminth infection

Pedro Gazzinelli-Guimaraes (LPD/NIAID/NIH)

Interleukin (IL)-11, a pleiotropic IL-6 family-member cytokine, has been described to play a role in both innate and adaptive immune responses and tissue inflammation. Little is known, however, about its function in helminth infection. Having shown that IL-11 gene expression is upregulated in Ascaris-infected lungs during the peak of larval migration at 8 dpi, we sought to understand the role played by IL-11 at lung barrier following Ascaris sp. infection in mouse models. Compared to uninfected mice, IL-11 levels were significantly elevated in the lung tissue of Ascaris-infected mice at 8 dpi (2,196 pg/mL vs 968 pg/mL, $p<0.001$). Mining of publicly available single-cell RNA sequencing data from mouse lungs followed by further validation using both flow cytometry and confocal microscopy, we found that EpCAM+ epithelial cells were the primary source of IL-11 following Ascaris infection. To assess the function of IL-11 in pulmonary helminth infection, we administered anti-IL-11 antibody intranasally during Ascaris infection which markedly impaired the influx of neutrophils to the lungs driven by Ascaris larvae. Moreover, Ascaris infection in IL-11Ra1 deficient mice induced a marked reduction in the

neutrophil's influx to the lungs (20.3 x 10^5cells vs 51.2 x 10^5cells, p=0.030), and a significant downregulation of CXCL-1 and G-CSF levels when compared with infected WT animals. To model the interaction between Ascaris, IL-11, neutrophils, and lung epithelial cells, we used an in vitro system whereby a human bronchial epithelial cell (HBEC3-KT) line grown in a monolayer on an extracellular gel matrix was shown to produce markedly increased amounts of IL-11 following exposure to Ascaris larvae. Moreover, in vitro stimulation of HBEC3-KT with rIL-11 induced marked levels of CXCL8, an important chemoattractant for human neutrophils. Taken together, our data suggests that IL-11 regulates the neutrophil-dominated inflammation in response to epithelial damage during acute helminth infection in the lungs.

Session 6. Cellular and Molecular Innate Immune Responses

Helminth infection as a treatment for vitiligo in mouse model

Hanchen Li (University of Massachusetts)

Vitiligo is an acquired T cell-mediated organ specific autoimmune skin disorder affecting roughly 1% of the population worldwide. It is characterized by the destruction of epidermal melanocytes histologically and white patches over the body clinically. Currently, there are no FDA-approved medical treatments to cure the disease. Helminths or their derived products have been shown to decrease pro-inflammatory responses and ameliorate symptoms in various autoimmune disease like multiple sclerosis, type I diabetes, rheumatoid arthritis, and inflammatory bowel disease. In vitiligo, it has been confirmed by multiple approaches that autoreactive CD8+ T cells specifically target and destroy melanocytes, eventually leading to depigmenting skin lesions. To study vitiligo in mice, sub-lethally irradiated mice with black skin and hair (KRT14-Kitl* transgenic mice) are adoptively transferred with melanocyte-specific CD8+ T cells (Pmels) and prime with a vaccinia virus (VV) vaccine, or with dendritic cells the same day. Such mice were divided into four groups, unifected control, infected with Heligmosomoides polygyrus, or infected with two different inocula of Trichuris muris. Infection occurred the day following irradiation. Beginning at approximately 4–5 weeks after disease induction, depigmentation was scored blindly at four anatomical sites including the tail, feet, nose, and ears. After final clinical scoring at week 8, the tail skin, spleen and skin-draining lymph nodes were processed into single cell suspensions and CD8+ Pmels were analyzed by flow cytometry. As compared to the control group, the Heligmosomoides polygyrus infected group showed a significant reduction of disease score, which is consistent with the Pmels numbers infiltrated in the epidermis and dermis. While the two Trichuris muris infection groups of mice were not significantly different from the control group. Here, we will provide the first known study of how parasitic nematode infections positively affect this autoimmune disease vitiligo, providing hope for future therapeutics based on nematode parasites.

CD8+ T cells provide protection during Cryptosporidium Infection

Breanne Haskins (University of Pennsylvania)

Cryptosporidium is a pathogen that resides in an intracellular, yet extracytoplasmic, location in intestinal epithelial cells and is an opportunistic pathogen in patients with defects in T cell-mediated immunity. To study the T cell response to Cryptosporidium, these parasites were engineered to express the model antigen SIINFEKL. This allows us to use transgenic OT-I CD8+ T cells specific for SIINFEKL to track Cryptosporidium-specific CD8+ T cells. The use of IFNγ-Thy1.1 reporter mice showed these parasites induced a potent IFNγ response from endogenous SIINFEKL-specific CD8+ T cells and activated a CD8+ T cell response which enhanced parasite control. To study how CD8+ T cells are primed, OT-I T cells that express a Nur77-GFP reporter of TCR activation were labeled with Cell Trace Violet and then transferred to infected mice. These studies revealed that OT-I cells were first exposed to antigen and proliferated (based on loss of CTV in the mesenteric lymph nodes (mLN)), while OT-I cells were not present in the small intestine until later during infection. To determine if cDC1s, the DC subset classically thought to prime CD8+ T cells, were important during Cryptosporidium infection, IRF8+32-/- mice were infected and the CD8+ T cell response quantified. Mice that lacked cDC1s were more susceptible to infection and had decreased CD8+ T cell responses. Thus, CD8+ T cells are primed in the mLN and cDC1s are critical for the generation of robust CD8+ T cell responses required for resistance to Cryptosporidium.

Cellular dynamics of immune evasion during Leishmania major infection

Romaniya Zayats (University of Manitoba)

Despite the generation of a strong T cell response, clearance of Leishmania major is incomplete and leaves a pool of chronically infected cells. Leishmania major driven induction of the immunosuppressive microenvironment through recruitment of regulatory T cells at the site of infection has been proposed to prevent parasite clearance. Here, we used a novel TCR transgenic mouse model, where CD4+ T

cells recognize an immunodominant peptide derived from Leishmania-glycosomal phosphoenolpyruvate carboxykinase (PEPCK), to visualize the dynamics of anti-L. major CD4+ T cell responses and to characterize mechanisms which restrain their effector function. We show that macrophage:T cell interaction dynamics were transient at steady-state, but prolonged upon antigen recognition. This activation leads to a production of high levels of IFNγ and can be significantly suppressed by PEPCK-specific Tregs in vitro, as compared to non-specific Treg controls. Co-culture of PEPCK-specific CD4+ T cells, L. major-infected macrophages, and Tregs shows that antigen activation leads to a substantial increase in IL-10 levels, while decreasing IL-12, TNF, and IL-2 production in the culture. Intravital microscopy studies characterizing PEPCK-specific CD4+ T cell migration dynamics and tissue localization within skin lesions directly in live mice show a significant recruitment of adoptively transferred effector T cells to the lesion site in vivo, displaying cellular behaviors consistent with antigen recognition at early and late stages of infection, yet cellular dynamics are augmented at the chronic stage, indicating a fundamentally altered environment. Upon secondary challenge with killed L. major, Tregs rapidly expand at the site of infection in healed mice. Currently we are evaluating how these Tregs are augmenting the cellular dynamics of Th1 cells in vivo. Collectively, our findings show for the first time that Leishmania-specific Tregs influence effector CD4+ T cell responses and this could be a mechanism that derives antigen persistence in L. major infection.

Sex-specific alterations in B cell development in a Maternal Schistosomiasis Model

Lisa Gibbs (University of Utah)

Maternal helminth infections are a global public health concern that correlate with altered infant immune responses to childhood immunizations and infection. A mechanistic understanding of how maternal infection and inflammation alters the immune responses of offspring is lacking but is critical to decrease childhood morbidity and to understand the consequences of specific long-lived immunity defects. Using our model of maternal Schistosoma mansoni infection, we have shown that murine pups born to mothers chronically infected with Schistosoma mansoni have reduced responses to vaccinations, corresponding to what has been reported in humans. To determine the origin of this humoral immunity defect, we began investigating the plasticity and functionality of progenitors of lymphoid cells critical for a protective humoral response. We found an increase in the common lymphoid progenitors (CLPs) in the bone marrow of pups from Schistosome infected mothers, but a marked decrease in immature and transitional B cells in male mice, indicating sex-specific limitations during B cell maturation. Using single cell V(D)J sequencing, we found only male pups from infected mothers have a more restricted repertoire compared to male mice from uninfected mothers after immunization, leading to decreased antigen-specific B cells in the germinal center of the draining lymph node. We have identified increased methylation on key immune regulatory genes that we believe underlies the mechanistic root of long-lived defects in humoral immunity to foreign antigens during maternal Schistosomiasis.

Th2 cells integrate into the tuft-ILC2 circuit to provide protective immunity to helminth infection

Alison Stanbery (University of Washington)

Both helminth infection and allergic responses involve type 2 immune activation at barrier tissues. Type 2 immune activation is characterized by cell recruitment, mucus production and tissue remodeling mediated by IL-13-secreting ILC2s and T helper 2 (Th2) cells. When activated chronically by allergens, tissue-resident Th2 cells (Th2 Trm) can cause morbidity, while ILC2s and circulating Th2s play important roles in clearing helminth infection. During acute helminth infection in the small intestine, epithelial tuft cells secrete IL-25 to activate ILC2s. ILC2-derived IL-13 acts on epithelial crypt progenitors to promote differentiation and increased frequency of tuft cells, thereby establishing a feed-forward tuft-ILC2 circuit that mediates epithelial remodeling and worm expulsion. It is unknown if Th2 cells can integrate into this circuit. Here we show that tuft cells contribute to Th2 Trm generation, and that Th2 Trm regulate tuft cell frequency. Using an in vivo model to permanently label and track cytokine-producing Th2 cells, we found distinct populations of lineage-traced Th2 Trm within intestinal and peripheral tissues. In particular, intestinal Th2 Trm express the receptor for IL-25, while Th2 Trm in adipose tissues express the IL-33 receptor. Loss of tuft cells results in a defect in the generation of Th2 Trm, leading to greater worm burdens. Finally, we found that helminth-induced Th2 are necessary for tuft cell hyperplasia in an antigen-dependent manner during chronic primary infection and are sufficient to induce tuft cell expansion during reinfection. Together, our data indicate that the tuft-ILC2 circuit can be rewired to incorporate Th2 cells and provide the first evidence that intestinal tuft cells contribute to adaptive immunity to helminths.

Crosstalk between microbiota and adaptive immunity determines susceptibility to amebic colitis

Md Jashim Uddin (University of Virginia)

Amebic colitis is caused by the pathogenic parasite Entamoeba histolytica. E. histolytica is also a causative agent for childhood diarrhea in the setting of poor sanitation and hygiene. Previously we demonstrated the critical role of the microbiome and innate lymphoid cells type 2 (ILC2) protecting from amebic colitis. Here we explore the role of the adaptive immune system in microbiome-mediated protection. Using the mouse model of amebic colitis, we observed that while wildtype (WT) C57BL/6J mice were resistant, RAG2-/- mice lacking an adaptive immune system were susceptible to acute amebic infection. We hypothesized that the lack of T and B cells shapes microbiota in a way that fails to provide resistance to colonization by amebic trophozoites in mice. To test that hypothesis, we performed a bedding swap between RAG2-/- and WT mice. WT mice that received bedding from the RAG2-/- cage were significantly more susceptible to amebic infection (17/19 vs. 6/20, p=0.0002). We performed a multiplex Luminex assay to characterize cytokines in colonic tissue lysate. Mice that received bedding from the RAG2-/- cage had increased type 3 cytokines (IL-6 and IL-22) and decreased type 2 cytokines (IL-4, IL-5, and IL-15). These data suggested that microbiota from RAG2-/- mice might increase susceptibility to amebiasis by inhibiting ILC2, as ILC2 orchestrate the protective type 2 immune response to amebic colitis. We are currently characterizing the ILC populations by scRNAseq to delineate the contribution of ILC2 to acquired immune system protection via the microbiome. We are also determining the microbial community that is associated with increased susceptibility to amebiasis and decreased type 2 immunity. In conclusion, our data reveal that the adaptive immune system may act in part to protect from acute amebic colitis via a microbiota-directed type 2 innate immune response.

POSTERS

P.1

Phenotypic diversity of serologic responses to Onchocerca volvulus in Cameroon: deciphering the genetic diversity for delineation of transmission zones

Linda Djune Yemeli (Centre for Research on Filariasis and other Tropical Diseases)

Onchocerciasis (river blindness) elimination mainly relies on annual or multi-annual preventive chemotherapy to interrupt the life cycle of Onchocerca volvulus (Ov) the causative agent of river blindness. Although this strategy has led to substantial reduction of onchocerciasis-related morbidity, a sustainable elimination of the disease requires a better understanding of geographic and thus genetic boundaries of different parasite populations to ensure that parasite will not be re-introduced in areas were elimination has been successful trough vector or human migration from areas of active transmission. However, tools to delineate onchocerciasis transmission zones/assess parasite re-introduction are scanty (likely because of the difficulty to obtain parasite materials) and the Ov population diversity is yet to be elucidated. Recently, it was demonstrated that difference in immunoreactivity to antigenic peptides' variants might be a useful tool for the characterization of Ov populations, thus making possible the study of Ov population diversity. We would therefore like to test whether immunophenotyping correlate with geographic areas and/or level of endemicity to onchocerciasis in Cameroon, since the prevalence of infection remains unexpectedly high in some foci despite decades of elimination efforts. Bio-banked sera (n=388) from Centre, Littoral and West Regions of Cameroon will be tested for their reactivity against the different variants of the 16 major immunogenic proteins of Ov using Enzyme-Linked Immunosorbent Assay. We will quantify the relative antibody response and use interclass correlation coefficient to determine whether there is a significant clustering of antibody levels by geographic areas and/or level of endemicity to Ov. We are expecting to decipher the phenotypic diversity of serologic responses to Onchocerca volvulus. These preliminary data will be helpful for a prospective study on immuno-characterization of Ov parasite population in Cameroon, with the ultimate goal to develop a serological method to delineate onchocerciasis transmission zones useful in a context of elimination.

P.2

Clinical, parasitological and molecular profiles of Cutaneous Leishmaniasis and its associated factors among clinically suspected patients attending Borumeda Hospital, North-East Ethiopia

Habtye Bisetegn (Wollo University)

Background: Cutaneous leishmaniasis is one of the most neglected tropical diseases increasing in its public health importance. In Ethiopia over 28 million people are living at risk of infection. Method: Institution based cross-sectional study was conducted at Borumeda Hospital from February to May 2019. A total 205 leishmaniasis suspected patients were included by systematic random sampling technique. Socio demographic characteristics were collected using pre-tested questionnaires. Parasitological investigation was done from skin slit sample by using Geimsa staining method. Species identification was done by PCR-RFLP. Data were entered in to EpiData version 3.1 and analyzed using SPSS version 20 software. P-value ≤0.05 was considered as statistically significant.

Result: A total of 205 participants consisting 59% male and 41% female included in this study. The mean age (±SD) of the study participants was 31.9 (±14.29). The overall prevalence of cutaneous leishmaniasis was 22.4% (46/205). The prevalence in males (13.7%) was higher than in females (8.8%). It was more prevalent in the age group 16-45years old (15.6%). Clinically, 60% of patients' hade single lesion with 1.55 average number of lesions. About 30.7% of patients had indurated plaque type of lesion. Most of the lesions were found on head and face (59%). House near to farmland, presence of hyrax in the village and presence of other cutaneous leishmaniasis cases in the neighborhood were independent predicator of cutaneous leishmaniasis prevalence. L. aethiopica was found to be the etiologic agent of cutaneous leishmaniasis in the study participants.

Conclusion: The prevalence of cutaneous leishmaniasis was 22.4%, this alerts the need of intervention. It is statistically associated with house near to farm land, presence of other cutaneous leishmaniasis cases in the neighborhood and presence of hyrax in village. Head and face were the most common sites of lesion.

P.3

Characterization of the molecular immunobiology of resistance or susceptibility to Schistosoma hematobium and Plasmodium falciparum co-infection

Tendai Makwikwi (Tshwane University of Technology)

Most neglected tropical diseases do not have vaccines and current control strategy available is use of preventive chemotherapeutic control, vector control and health education. Co-infections of malaria and schistosomiasis are common in areas where P. falciparum and

S. hematobium species are both also endemic. Single nucleotide polymorphisms within the promoter and intronic regions of cytokine genes have been associated with altered levels of circulating cytokines. The primary aim of the study was to investigate the association of the cytokine SNPs which are involved in differential cytokine production among S. hematobium and asymptomatic P. falciparum co-infected children aged between 6 – 13 years. A school-based epidemiological study was undertaken. A total of 986 children between 6 to 13 years were recruited into the study. Field work activities were undertaken. Of the 986 samples analyzed, 38.3 % were found to be infected with S. hematobium. Among the children, 27 % were infected with P. falciparum; while S. hematobium and P. falciparum co-infection was 9.9 %. No cases of S. mansoni and STHs were recorded from study areas. IgG4 levels were elevated in infected children. IgG1 was highly elevated in the 11 to 13 years age group which had the least infection. IgE was highly elevated in the uninfected children. CRP and resistin were elevated in S. hematobium infected children. The study demonstrated value in the field of immunobiology where it will help researchers working on the development of schistosomiasis and malaria vaccines in order to minimize infection and re-infection rates. Findings of this study clearly demonstrated that S. hematobium was prevalent in the area whereas P. falciparum was meso-asymptomatic. The study highlighted that infections in Africa do not exist singly, there is co-existence of S. hematobium with P. falciparum. SNPs in the cytokine genes do affect resistance or susceptibility to parasitic infections.

P.4

Effect of genetics and environment on immune composition and responsiveness in rewilded mice

Oyebola Oyesola (National Institutes of Health)

Immune responses during helminth infection, which currently affect over 2 billion people worldwide, can be varied with some individuals inducing optimal protective responses while others do not. The host genetic profile, environment and previous microbial experience could influence an individual's immune response, but the relative contribution, and interactions of these different factors remains largely unknown. Here, we assess the contribution and interaction of host genotype and environment to the immune cell landscape by releasing genetic inbred strains of mice, 129-SL, PWK and C57/B6 mice, to a rewilded environment and then infecting them with a murine intestinal helminth parasite, Trichuris muris. We find that immune composition and responsiveness are driven by interactions between genetics, environment and Trichuris muris infection status. Contrary to our initial hypothesis, we found that genotype has a stronger effect than environment and infection on both immune composition and cytokine response. However, genetic differences in immune variation can be reduced by environmental change. These findings suggest that genotype may be more important than the environment in determining the inter-individual variation of immune responses, although our model also indicates that interactions between genetics, environment, and infection is important at determining immune variation

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P.5

Host tissue proteomics reveal insights into the molecular basis of Schistosoma haematobium-induced bladder pathology

Derick Osakunor (Children's National Hospital)

Schistosomiasis remains a major public health concern worldwide. In the case of urogenital schistosomiasis (caused by Schistosoma haematobium), adult worms produce eggs in the bladder tissue, which are excreted in the urine and are the major cause of pathology. Disease manifestations include inflammatory fibrosis of the bladder, bladder carcinogenesis, and obstructive renal failure, with death rates of >150,000/year. While there are enormous opportunities to influence health through specifically designed interventions, limited mechanistic studies imply that the molecular mechanisms underlying pathology have not been well-defined. Based on a mouse bladder wall injection model for urogenital schistosomiasis, proteome profiling of schistosome-infected and uninfected mice bladder tissue was carried out to elucidate the pathways involved in pathology from urogenital schistosomiasis. Purified S. haematobium eggs (3,000 eggs/50 µl PBS) or uninfected hamster liver/intestinal extracts (controls) were microinjected into the bladder wall of mice. Mice were sacrificed 7 days post-injection, and bladders were lysed and processed for proteome profiling using mass spectrometry. Multivariate analyses in combination with protein-protein interactions and functional analysis showed that oxidative stress, cell adhesion/aggregation, coagulation, smooth muscle proliferation, and tissue regeneration proteins were more highly represented in egg-injected mice. Proteins involved in immunity/defence responses, including leukocyte migration/aggregation, inflammation, and response to interleukins were more prevalent in egg-injected mice. These findings highlight the host responses induced by active S. haematobium infection, characterised by several inflammatory and characteristic bladder tissue changes that lead to pathology, host and parasite survival. This study provides an in-depth analysis of potential host protein indicators and provide new insights into the pathophysiology of urogenital schistosomiasis and will be relevant for development of improved interventions for disease control.

P.6

Deficiency in astrocyte CCL2 production reduces immune cell infiltration and host defense in the CNS during chronic, but not acute, Toxoplasma gondii infection

Stephanie Orchanian (University of California, Irvine)

Toxoplasma gondii is an obligate intracellular parasite and a leading cause of death attributed to foodborne illness. T. gondii is a unique pathogen due to its ability to invade across the blood-brain barrier and persist for the remainder of the host's lifespan. CCR2+ immune cells play a key role in host survival during both acute and chronic T. gondii infection. These inflammatory immune cells are recruited to the meninges within 7 days and to the brain within 15 days post-infection (dpi) and persist during chronic infection. CCL2, a chemokine necessary for host survival during T. gondii infection, binds to the chemokine receptor CCR2 on monocytes and T cells. To examine the role of astrocyte-derived CCL2 on immune cell recruitment and pathogenesis in the brain, control CCL2fl/fl or GFAP-Cre x CCL2fl/fl mice, in which astrocytes are deficient in CCL2 expression, were infected with T. gondii and analyzed. In the GFAP-Cre x CCL2fl/fl mice, meningeal inflammation was decreased, monocyte, T cell, and neutrophil recruitment to the brain were reduced, and parasite cyst burden was increased during chronic T. gondii infection, indicating a critical role for astrocyte CCL2 in immune protection against chronic infection. In contrast, deficiency in astrocyte CCL2 production had no effect on immune cell infiltration during acute T. gondii infection. Surprisingly, microglia and infiltrating myeloid cells, and not astrocytes, were found to be the predominant producers of CCL2 at 15 dpi. These cells expressed elevated levels of CD68 and were enriched at sites of actively replicating T. gondii. This study sheds light on the effects of infection on immune cell infiltration of the meninges and brain and identified CCL2 production by microglia, infiltrating monocytes, and astrocyte as drivers for immune cell recruitment and host protection during T. gondii infection.

P.7

The Non-immune function of immune cells in skin fat homeostasis

Edries Hajam (Institute For Stem Cell Science and Regenerative Medicine)

We all get injuries from minor cuts, accidents, or surgeries. Our skin is very efficient in restoring the barrier upon injuries. However, we develop scars post injury. Scars are formed when connective tissue-producing cells; fibroblasts are chronically activated post-injury. Similar to scaring, in scleroderma (an autoimmune disease) activated fibroblasts over-produce connective tissue leading to skin dysfunction. Currently, there is no treatment of scaring or scleroderma and achieving scarless healing is every dermatologist's dream. Recent data suggest that skin fat cells can shrink and convert into activated fibroblasts. Thus, I am interested to understand how can we prevent the shrinking of skin fat cells thus preventing scaring/scleroderma. In both scaring as well as scleroderma the immune cell numbers are increased and the skin fat layer is decreased. Thus, I hypothesized that inflammatory cells may instruct skin fat cells to shrink. To investigate this hypothetical link, we employed a mouse model with skin inflammation. Strikingly, we observed a complete loss of skin fat layer. We also found the transfer of immune cells was sufficient to induce loss of skin fat. Mechanistically we found that CD4+ T and Macrophages create an inflammatory microenvironment prior to the loss of skin fat and signal skin fat cells to activate catabolism of triglycerides. Thus, we found a novel non-immune function of immune cells in controlling skin fat in the mouse model of inflammation. Lastly, we tested this pathway during homeostasis and found that immune cells instruct fat cells to shrink during homeostatic cyclic changes as well.

P.8

A unified signature of the host response to malaria revealed by transcriptomics meta-analysis

Pedro Souza (Federal University of Goiás)

Host-based gene expression analysis has been widely used to classify patients with malaria, identify diagnostic and prognostic biomarkers, and obtain insights into mechanisms of disease pathogenesis. However, confounding factors, such as age, sex, immune status, sample size, and gene expression technology exert significant influences on the data based in single and even large cohorts. This results in low reproducibility and limits its applicability to the clinical practice. We hypothesized that analysis of multiple cohorts incorporating both biological and technological heterogeneity would reveal a robust signature of human response to malaria. We used public transcriptome datasets from 9 different cohorts spanning 385 samples of whole blood or mononuclear cells. The cohorts were divided into discovery (n = 275) and validation (n = 110) datasets, obtained from 3 different microarray platforms. The meta-analysis of the 6 discovery cohorts (8131 genes), each one composed of patients with malaria and controls, revealed a differential expression of 329 genes (FDR adjust $p < 0.01$) involved with response to metal ions, innate immune system, interferon signaling, neutrophil degranulation, among other processes. Further analysis using a leave-one-dataset-out approach uncovered a robust signature of 9 up and 52 downregulated genes (FDR adjust $p < 0.01$), including SERPING1, CD59, NLRP1 and IL11RA. Receiver operating characteristic (ROC) analysis showed an expressive discriminative power to classify patients with malaria or healthy controls, with an overall area under the curve (AUC) of 0.95 (varying from 0.89 – 1 for discovery datasets). ROC analysis of 3 independent datasets resulted in AUCs of 0.95 for discriminating malaria

from healthy controls; 0.98 from malaria and convalescent patients; and 0.95 from malaria and cured patients. We identified a hostbased transcriptional signature displaying high power to discriminate patients with malaria from convalescent, cured and healthy individuals, which can be explored to improve malaria diagnosis and prognosis after treatment.

P.9

Hookworm adaptation to Type 2 immune responses

Annabel Ferguson (University of Pennsylvania)

Hookworms cause morbidity in humans and animals across the globe. The rising incidence of anthelmintic resistance and lack effective vaccines makes it imperative to generate new avenues for therapeutic intervention. Most infections are recurrent and hookworm burdens increase with host age in endemic regions, implying that hookworms adapt to host immune selective pressure. Interleukins 4 and 13 are central to host protective immunity against the rat hookworm Nippostrongylus brasiliensis (Nb). IL-4 and IL-13 signaling requires expression of the prototypical Type 2 transcription factor, signal transducer and activator of transcription 6 (STAT6) that drives expulsion and killing of worms through hematopoietic (TH2 cells, ILC2, M2 macrophages) and non-hematopoietic cells (Tuft cells, goblet cells, smooth muscle). This study addresses whether hookworms transcriptionally adapt to survival in STAT6 deficient hosts through changes in parasite fitness. We have assessed parasite fitness through quantification of eggs per gram of host feces (EPG), adult parasite hemoglobin levels, and individual adult parasite egg output. Data show that Nb shows improved fitness within STAT6 KO mice but when STAT6 KO adapted worms are introduced into WT mice, they show attenuated fitness. This altered fitness phenotype of STAT6 KO adapted parasites is reflected by a significant change in parasite transcriptional profile. Specifically, 7 transcripts are significantly up-regulated and 8 are down-regulated in STAT6 KO adapted parasites relative to WT lines. The most up-regulated transcript encodes a cysteine-rich/antigen 5/pathogenesis-related 1 CAP-family domain containing protein (9.3 log2 fold-change) implicated in hemoglobin degradation. Moreover, STAT6 KO adapted parasites elicit stronger host immune responses than WT passaged lines when inoculated into WT hosts. Collectively, this suggests that hookworms adapt to host immune pressure, which if lost, results in parasites with higher reproduction and feeding capacity, but greater immunogenicity. The mechanistic nature the adaptive change observed is under current investigation.

P.11

Assessment of Immunomodulatory, Toxicological and Antimalarial Effects Carapa Procera and Alchornea Cordifolia In Murine Models

Ayisha Mahama (University of Ghana)

Host-directed therapies primarily boost the host immune system against a range of diseases and also eliminate drug pressure that drives the development of drug resistance. Herbal plants are a cheaper, more accessible and, multi-component alternative to synthetic drugs. In this study, fractions of Carapa procera and Alchornea cordifolia, were investigated for immunomodulatory, toxicological and anti-malarial effects in murine models. To establish pre-clinical safety profiles, 14-day acute and 28-day sub-acute studies in Sprague Dawley rats and ICR mice were conducted with 1:1 chloroform fraction of Carapa procera, 1:1 chloroform and 100% petroleum ether. Alchornea cordifolia fractions. A dosage of 2000mg/kg P.o was administered during the acute study and 100mg/kg, 300mg/kg, and 1000mg/kg during the sub-acute study. Animals showed no clinical signs of toxidromes and showed normal weight gain throughout the study. Serum biochemical analysis indicated no significant elevations in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and, alkaline phosphatase (ALP). Urea (URE), creatinine (CR) also showed no significant elevations. Histopathological examinations of the liver and kidney indicated reversible liver degeneration with the 1000mg/kg dosage of CPL 100% fraction. In-vivo immunomodulatory using flow cytometry and anti-plasmodial with microscopy showed CPL 100% mg/kg had the highest anti-plasmodial activity of 26.67% reduction. CPL 100% mg/kg also showed increased IL-10 levels among treated mice. These preliminary findings demonstrate that fractions of Carapa procera and Alchornea cordifolia may safe, in support of their use as antimalarials. It also demonstrates that Carapa procera has the potential to be used as an anti-inflammatory agent.

P.12

An immunoepidemiological survey of schistosomiasis-malaria coinfection among school children in Magba subdivision, west region of Cameroon

Mireille Sylviane Dongmo Nguepi (University of Buea)

In areas where malaria is endemic, it is common for Plasmodium-infected people to also suffer from a concurrent helminth infection. Malaria and schistosomiasis are associated with high morbidity and mortality in Cameroon where both types of parasites burden are still high. Helminths have repeatedly been shown to modulate the immune system of their host in order to survive. Whether a concurrent

schistosome infection of the host can affect his immune response to Plasmodium spP. co-infection is still debated. Anti-plasmodium antibodies IgG1, IgG3, and IgG4 have been shown to play a protective role against malaria pathology. Contrarily, susceptibility to malaria infection has been shown to increase with the absence of schistosomiasis treatment with praziquantel, indicating schistosomiasis predisposes individuals to malaria infection. Therefore, the need to investigate the effect of schistosomiasis on malaria for a better understanding of this controversy. In Magba in the Western Region of Cameroon, the presence of a dam is one of the factors that account for the presence of schistosomiasis, where malaria infection is present and hence co-infection. We therefore evaluate the possible immune mechanisms by which Schistosomiasis predisposes or protects against Malaria infection. The prevalence of co-infection (malaria and Schistosomiasis), single infection of schistosomiasis, and malaria in Magba subdivision were determined to be 33.5%, 23.9%, and 17.1% respectively and the parasites species responsible for schistosomiasis burden include S. haematobium and S.mansoni while the species responsible for malaria burden include P.falciparum, P.malariae and P. ovale. The IgG subclass (IgG1, IgG2, IgG3, and IgG4) levels in single and schistosomiasis- malaria co-infected children were examined by ELISA to determine the effect of schistosomiasis on malaria susceptibility. This study shows that S. mansoni is protective against malaria whereas S. haematobium predisposes an individual to malaria infection.

P.13

Impact of toxoplasma gondii-induced crash on Treg homeostasis

Zachary Lanzar (University of Pennsylvania)

Acute infection of mice with Toxoplasma gondii results in a collapse of the regulatory T cell (Treg) population. While the infection-induced collapse of Treg cells has been defined, the re-building of the compartment and the long term impact of the crash have not been studied. Tregs were originally believed to be a monolithic population of suppressor cells, but there is recognition that there are distinct subsets termed central and effector Tregs. At homeostasis, central Tregs (cTregs) are a relatively quiescent subset that is dependent upon IL-2, whereas effector Tregs (eTregs) are IL-2 independent and exhibit potent suppressive activity mediated by the production of IL-10.1-3 The studies presented here reveal that infection results in a reduction in the cTreg and eTreg population that correlates with the loss of IL-2 production (see REFS). As acute infection resolves there is a rebound of the Treg population that correlates with increased IL-2. However, the composition of the Treg population is now dominated by the eTreg subset and the production of IL-10. The implications of an eTreg dominated compartment is unknown, however our data does raise new questions: (1) Is there a developmental relationship between cTregs and eTregs? (2) What mechanisms drive re-population of a heterogenous Treg compartment? (3) What are the long-lasting effects that infections impart on the Treg compartment? We aim to investigate the developmental relationship of the two Treg subsets, interrogate the impact of various cytokines on Treg re-population, and determine how infection shapes the re-built Treg compartment.

P.14

Moroccan primary Leishmania major strains differently modulate infection profile, iNOS and IL-1β gene expression in mice

Dounia Darif (Hassan II University of Casablanca)

In Morocco, zoonotic cutaneous leishmaniasis (ZCL) caused by Leishmania major is a public health issue characterized by polymorphic clinical lesions. This clinical polymorphism could depend on the vector, parasite genetic background, and host immune response. The overall objective of our work was to determine whether genetic L. major diversity impacted the host immunopathology. Five L. major strains were isolated from CL patients' lesions during a field mission in 2 ZCL endemic provinces: MHOM/MA/2017/T18, MHOM/MA/2017/T15, MHOM/MA/2017/T13 from Tinghir, and MHOM/MA/2017/Z41, MHOM/MA/2017/Z04 from Zagora. Swiss mice were injected with 1 × 106 metacyclic promastigotes of these strains into the hind footpads. The lesion development was measured weekly by a metric caliper. Mice were sacrificed at weeks 3 and 13 post-Infection (pi). Draining lymph nodes (DLNs), spleens and livers were collected to detect the parasites' kinetoplast DNA (kDNA) by PCR (13A/13B primers) and to analyze inducible nitric oxide synthase (iNOS) and IL1-β gene expression by RT-qPCR.

The Swiss mice infected with these L. major strains showed infection patterns similar to resistant phenotype. They developed small lesions between the 2nd and 3th weeks pi that progressed slowly and stabilized between the 8th and 12th weeks pi. These mice exhibited different infection profiles through differences in lesions' development and stabilization durations, which were longer with MHOM/MA/17/Z04 strain. Leishmania kDNA was detected in 100% spleens and livers, and 90% DLNs of mice infected with MHOM/MA/17/Z04 strain. Furthermore, RT-qPCR analysis revealed a decreased iNOS and IL-1β genes expression in DLNs and spleens of mice infected with this same strain at 3 and 13weeks pi.

Swiss mice infected with these Moroccan L. major strains showed different infection and genes expression profiles, suggesting that these responses were related to Leishmania strains. Further studies are in progress to analyze these genes' expression in other organs, as well as their production.

P.15

Long Pentraxin 3 (PTX3) Regulates IL-17A Mediated Immunity to Secondary Leishmania major Infection

Gaurav Gupta (University of Manitoba)

Cutaneous leishmaniasis caused by several species of protozoan parasites belonging to the genus Leishmania is endemic to the Middle East, Asia, Latin and Central America and North Africa. The disease affects millions of people in these regions and it is estimated that over 1 million new infections occur each year. Currently, there is no approved vaccine against the disease because of poor understanding of the mechanisms that regulate disease pathogenesis and correlates of protective immunity. Recently, our group reported that long Pentraxin 3 (PTX3), a pattern recognition molecule that plays a critical role in inflammation, tissue repair and wound healing, contributes to disease pathogenesis by negatively regulating protective Th17 responses. Here, we show that PTX3 also regulates secondary immunity to experimental cutaneous leishmaniasis. Healed PTX3-/- mice displayed enhanced resistance to secondary L. major infection compared to their healed wild-type counterpart mice. This enhanced resistance was associated with higher frequencies of effector memory CD4+ T cells in the spleens and draining lymph nodes. Interestingly, healed PTX3-/- mice produced higher levels of IL-17A than WT counterparts following secondary L. major challenge although IFN-ɣ levels were comparable in both strains of mice. Collectively, our results show that PTX3 also regulate secondary immunity to cutaneous leishmaniasis.

P.16

Investigating the role of Staphylococcus aureus on neutrophil recruitment and wound healing in a cutaneous leishmaniasis infection

Victoria Lovins (University of Pennsylvania)

Cutaneous leishmaniasis (CL) is a parasitic infection that causes a variable spectrum of disease ranging from single, self-healing lesions to chronic, non-healing lesions despite treatment. While the factors driving lesion chronicity are not clear, it is evident that many of the most severe forms of the disease are caused by uncontrolled inflammation. In mice, chronic CL lesions are frequently characterized by increased neutrophil infiltration and accumulation and persistent activation of inflammatory cytokines which exacerbate tissue damage and delay healing. Our lab has shown that colonizing microbes on the skin can contribute to immunopathology in murine models, however the role of the skin microbiota in regulating lesion healing during Leishmania infection is unknown. We have found that human CL lesions are often colonized by the pathogen Staphylococcus aureus (S. aureus) which delays healing in a strain-specific manner. We have also observed that wounded mice infected by different S. aureus clinical isolates cultured from lesions of patients with CL differentially impact neutrophil recruitment and lesion pathology. Overall, my project aims to investigate the strain- specific effect of S. aureus on immunopathology and lesion healing during CL infection.

P.17

Genetic mapping reveals Nfkbid as a central regulator of humoral immunity to Toxoplasma gondii

Kirk Jensen (University of California, Merced)

Protective immunity to parasitic infections has been difficult to elicit by vaccines. Among parasites that evade vaccine-induced immunity is Toxoplasma gondii, which causes lethal secondary infections in chronically infected mice. Here we report that unlike susceptible C57BL/6J mice, A/J mice were highly resistant to secondary infection. To identify correlates of immunity to virulent type I strains, we screened the AxB;BxA recombinant inbred mouse panel which led to the identification of Nfkbid, a nuclear regulator of NF-κB that is required for B cell activation and B-1 cell development. Nfkbid-null mice (“bumble”) did not generate parasite-specific IgM and lacked robust parasite-specific IgG, which correlated with defects in B-2 cell maturation and class-switch recombination. Though high-affinity antibodies were B-2 derived, transfer of B-1 cells partially rescued the immunity defects observed in bumble mice and were required for 100% vaccine efficacy in bone marrow chimeric mice. Immunity in resistant mice correlated with robust isotype class-switching in both B cell lineages, which can be fine-tuned by Nfkbid gene expression. We propose a model whereby humoral immunity to T. gondii is regulated by Nfkbid and requires B-1 and B-2 cells for full protection.

P.18

Investigating the role of serotonin 2C in regulating helminth-induced inflammation

Hannah Federman (Rutgers)

A growing body of literature, including our work and that of others, has revealed that neuropeptides and neurotransmitters regulate helminth-induced inflammation. Helminth parasites, such as hookworms, can affect several host tissues, including the lung and intestines, where they promote strong type 2 cytokine responses that support antihelminth immunity and the remodeling of affected tissues.

Additionally, our work has recently identified monocyte-derived alveolar macrophages (Mo-AMs) as critical regulators of these host protective responses. Mo-AMs populate the lung in response to infection-induced inflammation and take on a highly activated and profibrotic phenotype. Further, our work has shown that Mo-AMs begin to change their phenotypes the longer they persist in the lung and receive tissue-specific signals. Despite these advances, whether neuron-derived signals participate in this tissue imprinting process remains unknown. To address this, we performed transcriptional profiling of lung macrophage populations following infection with the hookworm Nippostrongylus brasiliensis (Nb) and investigated their expression of various receptors for neuron-derived molecules. Interestingly, Mo-AMs were found to gradually acquire high levels of the serotonin 2C receptor (5HTR2c) after entering the lung microenvironment. Serotonin (also known as 5HT) is a neurotransmitter that plays important roles in myriad biological functions beyond its well described roles in the central nervous system. Serotonin is largely produced by enterochromaffin cells in the GI tract, whereupon almost 95% of it is taken up by circulating platelets. However, the role of the remaining serotonin in regulating helminth-activated macrophages remain unknown. Therefore, we sought to test whether 5HT can regulate the expression of profibrotic molecules by Mo-AMs. Our findings indicate that 5HT is sufficient to reduce the expression of Arginase 1 by monocyte-derived macrophages and provoke the hypothesis that serotonin inhibits the activation state of Mo-AMs to prevent excessive tissue remodeling post-Nb infection. This will be directly tested in future experiments by deleting 5HTR2c in specific macrophage lineages.

P.19

The matricellular protein Mindin induces pro-inflammatory response in fibroblasts to manifest dermal fibrosis

Gaurav Kansagara (Institute for Stem Cell Science and Regenerative Medicine-inStem)

Fibrosis is an inflammatory disease that can occur in any connective tissue of the body and can lead to loss of organ function. The key player in this disease condition is activated fibroblasts, which produce excessive amounts of ECM proteins that compromise tissue physiology. The entire process of fibrogenesis is multifactorial, as various secreted soluble factors mediate the persistent activation of fibroblasts. However, the aetiology of the disease is not yet well understood. Using a mouse model of skin fibrosis, we have identified a matricellular protein called Mindin that plays an indispensable role in fibrogenesis. Our data indicate that mindin increases migration, contraction, pro-inflammatory cytokine production and secretion of ECM proteins by dermal fibroblasts. Our current efforts are focused on elucidating the signalling pathway mediating the effects of mindin on fibroblasts. Our studies have demonstrated that the N-terminal(Mindin-FS) can recapitulate the effects of full-length mindin(Mindin-FL) on mouse fibroblasts. Fibrosis affects nearly every tissue in the body and contributes to 1/3rd of the deaths worldwide, yet, there is no effective cure available to combat fibrosis. Considering this enormous public health burden of fibrotic diseases, our studies have the potential to discover new pathways that can offer novel routes for therapeutic intervention.

P.20

The role of Dihydrolipoyl dehydrogenase (DLD) in the immunopathogenesis of L. major

Somtochukwu Stella Onwah (University of Manitoba)

Introduction: Cutaneous leishmaniasis (CL) caused by numerous Leishmania species including Leishmania major, leads to a range of diseases, from self-healing lesions to chronic disfiguring disease. Protection is achieved from IFN-ɣ producing CD4+ T cell activation on Leishmania infected macrophages. Dihydrolipoyl dehydrogenase (DLD) is a critical mitochondrial enzyme in eukaryotic cells including Leishmania known to modulate metabolic activities. In different pathogenic organisms, DLD is a promising therapeutic target. The role and contribution of DLD in L. major immunopathogenesis is currently not known. We hypothesize that DLD is a virulence factor and its deficiency in L. major will result in an attenuated disease pathology and altered host immune response.

Methods: To generate DLD deficient L. major, an all-in-one plasmid (pLDCN) containing two short oligonucleotide sequences (guide RNA) complementary to the DLD gene in L. major is introduced such that upon expression, Cas9 will initiate cleavage. A homology-directed repair mechanism allowed for the introduction of a donor DNA (bleomycin) PCR product into the deleted site. I functionally validated DLD gene deletion in axenic culture, bone marrow-derived macrophages and in mice.

Results: Deficiency of DLD in L. major was confirmed by PCR and in vivo by the absence of DLD-specific CD4+ T cells from splenocytes of mice infected with DLD deficient parasites using DLD-specific tetramers. Growth kinetics in axenic culture and macrophages show that deficiency of DLD gene products results in reduced proliferation in comparison to wild-type (WT) parasites. Mice infected with DLD deficient parasites in the footpad had no observable lesion and significantly reduced parasite burden compared to WT-infected animals. The frequency of cytokine (IFN-ɣ and TNF)-producing CD4+ T cells in spleens of mice infected with DLD deficient parasites was significantly lower than those from their WT counterparts. Cells from mice infected with DLD deficient parasites produced significantly reduced levels of these cytokines in the culture supernatant following in vitro restimulation with soluble Leishmania Ag.

Conclusion: These findings suggests that DLD in L. major is a critical metabolic enzyme for intracellular survival both in axenic culture and inside macrophages. Since DLD deficient parasites did not induce pathologies in mice but alters host immune response indicates that they could be good vaccine candidates.

P.21

Protective CD4+ Th1 cell-mediated immunity is reliant upon execution of effector function prior to the establishment of the pathogen niche

Leah S Hohman (University of Calgary)

Intracellular infection with the parasite Leishmania major features a state of concomitant immunity in which CD4+ T helper 1 (Th1) cell-mediated immunity against reinfection coincides with a chronic but sub-clinical primary infection. In this setting, the rapidity of the Th1 response at a secondary site of challenge in the skin represents the best correlate of parasite elimination and has been associated with a reversal in Leishmania-mediated modulation of monocytic host cells. Remarkably, the degree to which Th1 cells are absolutely reliant upon the time at which they interact with infected monocytes to mediate their protective effect has not been defined. In the present work, we report that CXCR3-dependent recruitment of Ly6C+ Th1 effector (Th1EFF) cells is indispensable for concomitant immunity and acute (<4 days post-infection) Th1EFF cell-phagocyte interactions are critical to prevent the establishment of a permissive pathogen niche, as evidenced by altered recruitment, gene expression and functional capacity of innate and adaptive immune cells at the site of secondary challenge. Surprisingly, provision of Th1EFF cells after establishment of the pathogen niche, even when Th1 cells were provided in large quantities, abrogated protection, Th1EFF cell accumulation and IFN-ɣ production, and iNOS production by inflammatory monocytes. These findings indicate that protective Th1 immunity is critically dependent on activation of permissive phagocytic host cells by pre-activated Th1EFF cells at the time of infection.

P.22

Th1/Th2 cross-regulation controls early Leishmania infection in the skin by modulating the size of the permissive monocytic host cell reservoir

Matheus Batista Carneiro (University of Calgary)

The impact of T helper (Th) 1 versus Th2 immunity on intracellular infections is attributed to classical versus alternative activation of macrophages leading to resistance or susceptibility. However, observations in multiple infectious settings demonstrate deficiencies in mediators of Th1/Th2 immunity have paradoxical or no impact. We report that prior to influencing activation, Th1/Th2 immunity first controls the size of the permissive host cell reservoir. During early Leishmania infection of the skin, IFN-ɣ- or STAT6-mediated changes in phagocyte activation were counteracted by changes in IFN-ɣ-mediated recruitment of permissive CCR2+ monocytes. Monocytes were required for early parasite expansion and acquired an alternatively activated phenotype despite the Th1 dermal environment required for their recruitment. Surprisingly, STAT6 did not enhance intracellular parasite proliferation, but rather modulated the size and permissiveness of the monocytic host cell reservoir via regulation of IFN-ɣ and IL-10. These observations expand our understanding of the Th1/Th2 paradigm during infection.

P.23

PGD2 and CRTH2 circuit during type 2 inflammation

Shuchi Smita (University of Washington)

Helminth infection elicits Type 2 inflammation that results in dramatic tissue remodeling. Upon parasite infection, intestinal epithelial cells (IECs) releases cytokines like interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP) that activate innate and adaptive immune cells that secrete Type 2 cytokines IL-4, -5, -9 and -13. IL-13 then circuits back to IECs to alter their differentiation and function. Tuft and goblet cell hyperplasia occurs, and increased mucin production by goblet cells, increased barrier permeability, and elevated smooth muscle contractility help to expel helminths from the host. Our recent findings show that a bioactive lipid, prostaglandin D2 (PGD2), and its receptor CRTH2 negatively the regulate Type 2 cytokine-driven epithelial program during murine helminth infection with Nippostrongylus brasiliensis. Loss of CRTH2 resulted in elevated goblet cell accumulation and accelerated worm clearance. New studies focus on the biological significance of the PGD2-CRTH2 pathway/ circuit during Type 2 inflammation and the consequences of loss of CRTH2 during and after infection. We hypothesize that the PGD2-CRTH2 circuit may play an important role in regulating the Type 2 inflammatory response to allow for a return to normalcy after helminth infection. Using novel Villin-Cre CRTH2flox GFP reporter mice, we delineate CRTH2-exprressing cells in the small intestine, including intestinal tuft cells, and show that IEC-intrinsic CRTH2 loss recapitulates the phenotype of whole-body CRTH2 knockout mice. In addition, after clearance of N. brasiliensis, mice that lack CRTH2 retained

inflammatory IEC changes, while the epithelium returned to a normal state in wild-type animals after infection was cleared. These preliminary findings suggest that CRTH2 plays a critical role in IEC-intrinsic regulation of the epithelial response to helminth infection and the return to homeostasis following infection.

P.24

A protozoan gut commensal remotely regulates an extra-intestinal eosinophil niche.

Arthur Mortha (University of Toronto)

The gut microbiome influences chronic inflammation of the airways via the gut-lung axis. However, causal connections between microbes and their host, including the underlying mechanisms for this phenomenon remain largely unknown. Here, we show that colonization with the gut commensal protozoa, Tritrichomonas musculis (T.mu), remotely shapes the lung immune landscape and exacerbates allergic airway inflammation. We demonstrate that colonization with T.mu mediates the T and B cell-dependent accumulation and activation of inflammatory group 2 innate lymphoid cells in the lungs to constitute a tripartite immune network that serves as a niche for lung eosinophils. Animals colonized with T.mu show severely exacerbated allergic inflammation in the airways and reveal a new protozoan-driven gut-lung axis that remotely shapes the lung immune network to potentiate chronic pulmonary inflammation.

Collectively, our data demonstrates, that a gut commensal protist, as a permanent member of the gut microbiome, changes the lung immune landscape across the gut-lung axis via engagement of a tripartite immune network of lymphocytes to exacerbate allergic airway inflammation.

P.25

Investigating the roles of the cell regulator TRIM24 during experimental visceral leishmaniasis

Edward Muscutt (University of York)

Visceral leishmaniasis (VL) is a neglected tropical disease caused by infection with protozoan parasites Leishmania donovani and L. infantum, with >95% cases fatal if left untreated. Current treatments are limited, expensive, and toxic, and there are currently no vaccines available. Macrophages are essential for the pathogenesis of VL. Leishmania parasites modulate macrophage signalling pathways to dampen leishmanicidal activity and facilitate persistence in an intracellular niche. However, the mechanisms behind this remain poorly understood. Recently TRIM24, a member of the tripartite motif protein family and a previously identified regulator of interferon STAT signalling, was predicted as an upstream negative regulator of host pro-inflammatory gene expression in a model of VL. In this study we investigate the roles of TRIM24 in macrophage activation and during L. donovani infection by utilising Trim24-deficient C57BL/6 mice. Comparing B6.Trim24-/- and wild type B6 bone marrow-derived macrophages (BMMs), we found that Trim24-deficiency was associated with increased expression of iNOS and release of nitric oxide. Despite the important role of NO in host protection, we did not observe any difference in parasite loads in vivo, as determined by bioluminescent imaging of infected B6 and B6.Trim24-/- mice. To further understand why Trim24-deficiency fails to lead to heightened host resistance (through elevated NO) we examined release of IFN-β, implicated in the negative regulation of immunity to L. donovani. BMMs from B6.Trim24-/- mice produced more IFN-β in response to LPS compared to wild type BMMs. Collectively, our data providing a potential mechanism whereby the positive (NO) and negative (IFN-β) effects of Trim24-deficiency are counter-balanced in vivo.

P.26

Alterations to the skin immune and stromal cell landscape following L. donovani infection in UVB-exposed hosts

Marcela Montes de Oca (University of York)

Stromal and immune cell interactions are critical in generating efficient immune responses against pathogens. This interplay of signals and co-ordination of functions exists in a delicate balance that can be perturbed by environmental stimuli, including UVB irradiation. Our knowledge of how UVB alters the immune and stromal landscape of the skin during Leishmania donovani infection is limited. While the immunosuppressive effects of UVB have been widely documented, transcriptional alterations in these key populations have not been reported. Using single-cell RNA sequencing, flow cytometry, histopathology, and in vivo bioluminescence imaging we show that UVB negatively regulates fibroblast reticular cells (FRCs), activated endothelial cells and macrophage/monocyte populations in the skin of L. donovani infected mice. Transcriptional profiling reveals infection specific changes in macrophage populations, compared to UVB treatment alone. Our study provides a cellular framework that identifies cell populations specifically affected by UVB during L. donovani infection. This framework serves to understand VL pathogenesis in the skin with the aim of developing targeted therapies to improve disease outcome and reduce transmission potential.

P.27

Determining the importance of CX3CR1+ cells during placental infections

Samuel Leeds (Purdue University)

The placenta is the only temporary organ of the human body and plays a crucial role in facilitating the transfer of nutrients and waste between a developing fetus and the mother. However, it also creates a site for pathogen transmission to the developing fetus. Placental macrophages are common targets for pathogens with placental tropisms. In humans these macrophages can be divided in to two groups, fetal derived Hofbauer cells and maternal decidual macrophages. However, the importance and role of the different macrophage subtypes during infection of the placenta is unknown. The chemokine receptor CX3CR1 is commonly found on resident macrophages, it is also expressed in both murine and human placentas and has been implicated in trophoblast and leukocyte migration. Additionally, several studies have shown a role of CX3CR1 in various models of inflammation in other tissues. The aim of this study is to determine the role of CX3CR1+ macrophages during infection in the placenta. Wild type mice were infected 12.5 dpg with 10¬4 T. gondii tachyzoites. At 5 dpi placentas were harvested and processed for either flow cytometry or immunofluorescent staining. Preliminary results from flow cytometry characterization reveled multiple CX3CR1+ cell populations within the uninfected placentas of Wt mice; this included a fetal and maternal macrophage populations and 2 maternal derived monocyte population among others that have yet to be fully characterized. Interestingly, nearly all of the macrophage subpopulations expressed CX3CR1, indicating an importance function of this receptor in regulating immune responses at the maternal-interface.

P.28

Current status and Hosts' Immune Response to Human African Trypanosomiasis in Nigeria

Maureen O. Efenovwe (University of Ibadan)

Background: Despite plans by the World Health Organization to eliminate of Human African Trypanosmiasis as a public health problem by 2030, challenges abound that may hinder this goal. Challenges which include limited sensitivity of current diagnostic techniques, existence of trypano-tolerant hosts and existence of animal reservoirs. The purpose of this study was to establish the current status of Human African Trypanosomiais and ascertain hosts' immune response in the outcome of the disease in communities in Delta and Ogun States in Nigeria.

Method: A total of 285 blood samples were collected and screened microscopically for the presence of T.b. gambiense. ELISA was done to determine the presence of T. b. gambiense IgG and IgM antibodies as well as the concentration of four cytokines (TNF-α, IL-1α, IL-10 and CXCL10).

Result: Only 1 sample (0.36%) was positive with microscopy, 61 (21.4%) and 42 (14.7%) were seropositive for T. b. gambiense IgG and IgM respectively, 12 (4.2%) were seropositive to both T. b. gambiense IgG&IgM. Mean CXCL-10 was significantly higher (P=0.043) in the IgM seropositives. Significantly higher (P=0.002) IL-10 was observed in samples that were positive with both IgG&IgM. There was no significant difference (P=0.759) in mean TNF-α concentrations in all groups and higher mean IL-1α was observed individuals with both IgG& IgM antibodies.

Conclusion: There is an on-going transmission of Human African Trypanosomiasis in Abraka Delta State and Eggua, Ogun State Nigeria. There was an up-regulation of IL-1α and IL-10 during antibody switching, thus making both cytokines possible indicators for late stage HAT.

P.29

Interrogating host-protective contributions of lung macrophages in response to helminths

John Ponessa (Ph.D. Candidate)

Helminth infections are a major cause of morbidity in the developing world and represent a significant public health concern. Although the clearance of worms and healing of helminth-induced wounds are dependent on type 2 immune responses, the pathways that regulate these processes remain to be fully defined. Previous work by our lab and others have demonstrated that alternatively activated (M2) macrophages are critical contributors to host-protective responses following a helminth challenge. For example, it has been demonstrated that M2 macrophage responses mediate reductions in parasitic burdens, regulate inflammation, and promote the healing of helminth-affected tissues. However, emerging studies now suggest that the M2 macrophages involved in these processes are a heterogenous population comprised of monocyte-derived macrophages (Mo-Macs) and tissue-resident macrophages (TD-Macs). Initially, these populations are easily identified and appear phenotypically and functionally distinct, however recent research has now revealed that Mo-Macs can convert to a tissue-resident-like phenotype over time. Despite these advances, the kinetics of this process and magnitude to which it occurs remains poorly understood. Here we use Cx3cr1CreER X R26TdT mice, which allow us to observe changes that occur as

monocyte-derived macrophages supplement tissue-resident macrophages and remain in the lung post-Nippostrongylus brasiliensis(Nb) infection. Our data demonstrate that monocytes enter the lung and undergo a gradual transition process as they adopt a tissue-resident-like phenotype and acquire substantial transcriptional changes between days 7, 14 and 30 post-Nb infection. Further, our studies reveal that Mo-Macs express type 2-associated markers and show a distinct remodeling of the chromatin landscape relative to TD-Macs. In particular, they express high amounts of arginase-1 (Arg1), which we demonstrate mediates helminth killing through L-arginine depletion. These studies indicate that recruited monocytes are selectively programmed to promote antihelminth responses.

P.30

Development and efficacy of various pan-hookworm vaccine targets

Jeffrey Chicca (University of Massachusetts)

Over 500 million people worldwide suffer from hookworm infections and disease. Despite years of concerted effort at deworming campaigns, hookworm infections remain prevalent due to imperfect anthelmintic efficacies. Development of a pan-hookworm vaccine would significantly impact hookworm disease by limiting primary infection and mitigating reinfections. Here we describe the identification and testing of potential targets suitable for vaccine development using two distinct approaches: 1) excretory/secretory (ES) products from the human zoonotic hookworm Ancylostoma ceylanicum and 2) hookworm protein candidates generated from RNA sequencing and cDNA data analysis of A. ceylanicum. Our vaccination experiments typically involve alum hydroxide in outbred Golden Syrian hamsters (Mesocricetus auratus) with A. ceylanicum challenge infections. Parasitic nematodes excrete or secrete various proteins and molecules (ES products) to establish and maintain infection. ES products from other parasitic nematodes are emerging as a potentially good sources of vaccine antigens. Here, we present data on the collection and characterization of A. ceylanicum ES products. Additionally, we evaluate the efficacy of ES products as a source of vaccine antigens. In parallel, the A. ceylanicum genome and transcriptome were screened for vaccine candidates in whole animals, dissected tissues, and hosts with varying immune systems. These analyses yielded target antigens, confirmed by cDNA analysis and RT-PCR, which we then tested in vivo in vaccination- challenge experiments. Target proteins were heterologously expressed and purified, injected three times into hamsters, and then subsequently challenged with hookworms. Here we discuss our results with over 10 target antigens, including at least one with promise for further vaccine development.