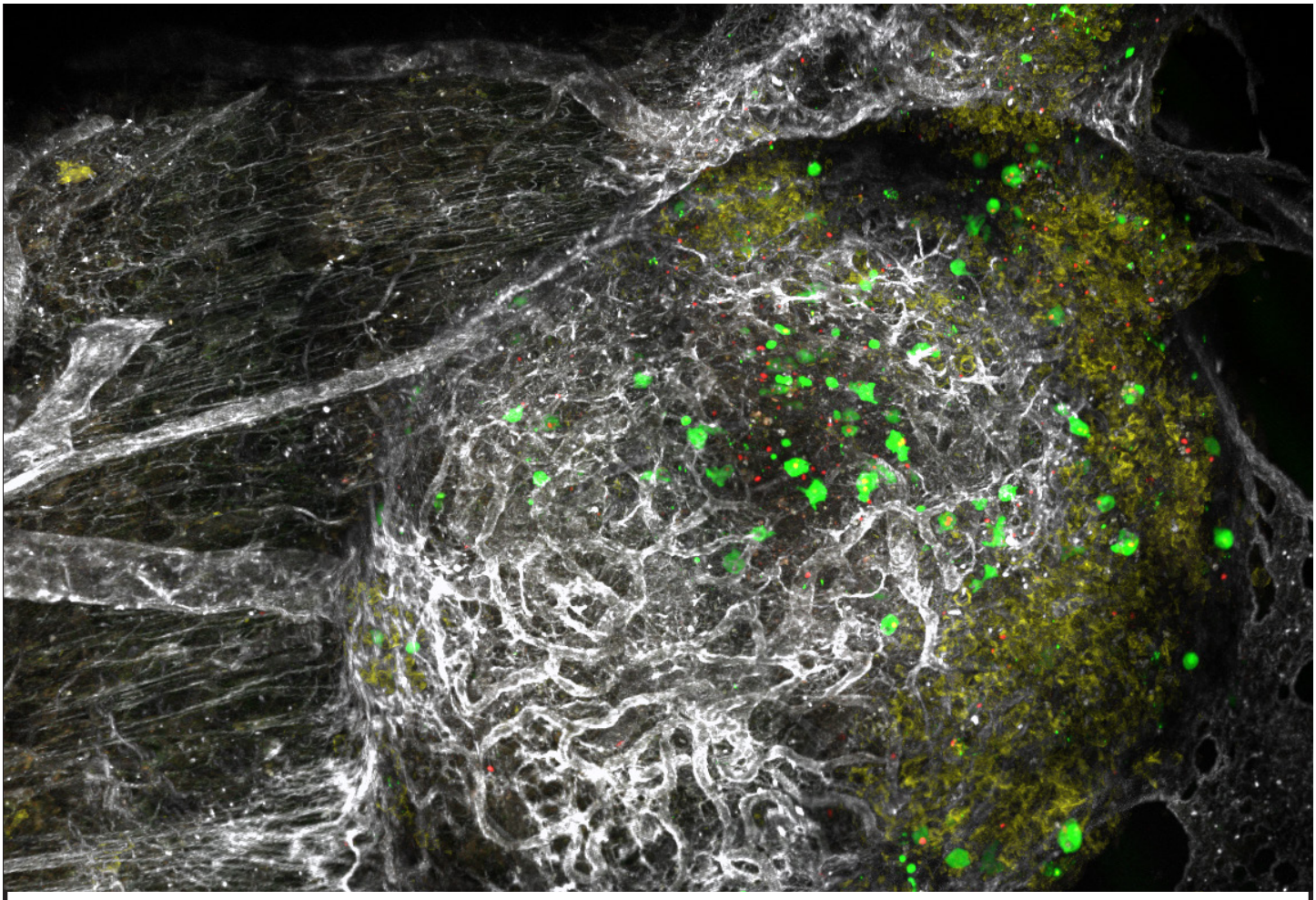


WHIP 2021

25th ANNUAL WOODS HOLE IMMUNOPARASITOLOGY MEETING

VIRTUAL

April 11 – 14, 2021

SCIENTIFIC PROGRAM

Sunday, April 11

11:00 AM – 11:15 AM **Welcome**
Elia Tait Wojno, Jude Uzonna

11:15 AM – 12:15 PM **Modulation of host responses and virulence by protozoan RNA viruses**
Dr. Steve Beverley

12:15 PM – 12:30 PM Break

12:30 PM – 2:15 PM **Session: Neuroimmunology**
Chairs: Drs. Tajie Harris & Jason Stumhofer

12:30pm Enteric helminth coinfection enhances host susceptibility to neurotropic flaviviruses via a tuft cell-IL-4 receptor signaling axis
Pritesh Desai, desai@wustl.edu

12:45pm P. berghei Infection Expands Microglia Populations Associated with Activation and Proliferation and Induces Upregulation in Inflammation Related Genes
Marshall Roedel, marshall.roedel@path.utah.edu

1:00pm Investigating astrocyte subsets during Toxoplasma gondii infection
Zoe Figueroa, zfigu001@ucr.edu

1:15pm Helminth-activated monocytes inhibit microglial activation and neuroinflammation
Jianya Peng, jp1331@njms.rutgers.edu

1:30pm Lymphatic drainage of cerebrospinal fluid regulates peripheral T cell responses during Toxoplasma gondii brain infection
Michael Kovacs, mk5bs@virginia.edu

1:45pm Tuft cell-derived acetylcholine regulates epithelial fluid and mucus secretion in Type 2 immunity
Tyler Billipp, tbillipp@uw.edu

2:00pm Beta-amyloid's antimicrobial properties protect elderly mice from Toxoplasma gondii infection in the face of age-related immunological decline.
Kathryn McGovern, kemcgovern@arizona.edu

2:15 PM – 3:30 PM **Social Event**

Monday, April 12

11:00 AM – 12:00 PM **Memory B cell responses to infection**
Dr. Marion Pepper

12:00 PM – 12:30 PM Break

12:30 PM – 1:45 PM **Session: B cells and humoral immunity**
Chairs: Dr. Arthur Mortha & Dr. Nathalie Steinel

12:30pm T follicular helper (Tfh)-germinal centre (GC) B cell response is required for sterile immunity during enteric helminth infection
Aidil Zaini, muhammad.binahmadzaini@monash.edu

12:45pm Characterization of intercellular communication between B cells exposed to Leishmania donovani
Tanja Stögerer, tanja.stogerer@inrs.ca

1:00pm Secondary antigen stimulation contributes to the heterogeneity of pathogen-specific CD8+ T cell response during acute and chronic toxoplasmosis
Lindsey Shallberg, lshall@pennmedicine.upenn.edu

1:15pm Dissecting the role of helminth antigen exposure in reprograming hematopoietic development
Lisa Gibbs, lisa.gibbs@path.utah.edu

1:30pm An invariant Trypanosoma vivax vaccine antigen inducing protective immunity
Gavin Wright, gw2@sanger.ac.uk

1:45 PM – 2:15 PM Break

2:15 PM – 4:00 PM **Session: Immune regulation, the microbiota, and host and parasite metabolism**
Chairs: Drs. Maritza Jaramillo & Oliver Harrison

2:15pm Staphylococcus aureus induces Trichuris muris egg hatching via a contact dependent
Amicha Robertson, amicha.robertson@nyulangone.org

2:30pm Small intestinal levels of the short-chain fatty acid isovalerate are elevated during Heligmosomoides polygyrus infection and can promote helminth fecundity
Mia Kennedy, mhekennedy@gmail.com

2:45pm Immunological dynamics of gastrointestinal parasitism: investigating the relationship between Trichuris muris trickle infection, the mucosal barrier, and the microbiota
Stefano Colombo, stefano.colombo@manchester.ac.uk

3:00pm Control of systemic eosinophilia by a protozoan commensal dictates asthma severity
Arthur Mortha, arthur.mortha@utoronto.ca

3:15pm Mechanisms of gut microbiota mediated expansion of granulocyte monocyte progenitors
Brandon Thompson, bat7n@virginia.edu

3:30pm Amino acid transport acts as a rheostat of ILC2 responses
Suzanne Hodge, suzanne.hodge@postgrad.manchester.ac.uk

3:45pm Breathing is Eating: A Toxoplasma Oxygen Sensor Mediates Virulence By Regulating Tryptophan Scavenging in Response to IFNɣ
Charlotte Cordonnier, ccordonn@buffalo.edu

4:00 PM – 4:15 PM Break

4:15 PM – 6:15 PM **Poster Session 1**

Tuesday, April 13

10:45 AM – 12:15 PM **Session: Technology, informatics, and unique models of disease**
Chairs: Drs. De'Broski Herbert & Lucy Jackson-Jones

10:45am Understanding granuloma heterogeneity in experimental visceral leishmaniasis through spatially-resolved multi-omic approaches.
Shoumit Dey

11:00am Using re-wilded mice to characterize the inferential power of ecoimmunological assays for describing immune phenotype
Alexander Downie, adownie@princeton.edu

11:15am Threespine stickleback as an emerging model for cestode-mediated immunomodulation
Natalie Steinel, natalie_steinel@uml.edu

11:30am Tick-borne co-infections and immune alterations during progression of canine leishmaniosis
Breanna Scorza, breanna-scorza@uiowa.edu

11:45am Condensed-barcoded gRNA libraries facilitate genome-wide screening of Toxoplasma during mouse infection
Chris Giuliano, giuliano@wi.mit.edu

12:00pm In vitro co-infection of Leishmania infantum and Borrelia burgdorferi produces inflammatory cytokines and increased Leishmania parasite burden
Danielle Pessôa-Pereira

12:15 PM – 12:30 PM Break

12:30 PM – 2:00 PM **Session: Cellular and molecular innate immune responses**
Chairs: Drs. Christine Petersen & Irah King

12:30pm Role of IL-33 in protection from colitis due to Entamoeba histolytica
Md Jashim Uddin, mu2qx@virginia.edu

12:45pm cGAS-STING pathway activation during Trypanosoma cruzi infection leads to tissue-dependent parasite control
Natasha Perumal, ai18242@uga.edu

1:00pm Mechanisms of inflammasome activation in cutaneous leishmaniasis
Christina Go, goc@upenn.edu

1:15pm Localized circuitries in cutaneous leishmaniasis that allow dermis resident macrophages to maintain M2-like properties in a strong Th1 environment
Sang Hun Lee, leesh2@nih.gov

1:30pm IgM is required for expansion of macrophages in the pleural cavity during Litomosoides sigmodontis infection
Lucy Jackson-Jones, L.jackson-jones@lancaster.ac.uk

1:45pm Trichinella spiralis-induced mastocytosis and erythropoiesis are simultaneously supported by a bipotent mast cell/erythrocyte precursor cell
Christina Hernandez, cmh329@gsbs.rutgers.edu

2:00 PM – 2:15 PM Break

2:15 PM – 4:00 PM **Session: T cell responses**
Chairs: Drs. Phillip Scott & Fernanda Novais

2:15pm Effector function prior to establishment of the phagosomal pathogen niche is required for protective CD4+ T cell-mediated immunity against Leishmania
Leah Hohman, leah.hohman@ucalgary.ca

2:30pm Transgenic T cell epitope-expressing Strongyloides ratti reveals that helminth-specific CD4+ T cells constitute both Th2 and Treg populations
Bonnie Douglas, bonniebd@pennmedicine.upenn.edu

2:45pm PD-L1 – PD-1 interactions limit effector Treg cell populations at homeostasis and during infection
Perry, Joe Perry, Joseph jallan@pennmedicine.upenn.edu

3:00pm The impact of antigen dose on the generation, function and longevity of Leishmania-specific CD4+ memory T cells
Zhirong Mou, zhirong.mou@umanitoba.ca

3:15pm Helminth-driven fetomaternal crosstalk primes regulatory networks to modify inflammatory T cell responses
Matthew Lacorcia, matthew.lacorcia@tum.de

3:30pm MicroRNA-21 deficiency promotes the early Th1 immune responses and resistance towards visceral leishmaniasis
Erin Holcomb, holcomb.149@osu.edu

3:45pm Heterogeneity of Tregs in Helminth Infection
Caitlin McManus, c.mcmanus.2@research.gla.ac.u

4:00 PM – 4:15 PM Break

4:15 PM – 6:15 PM **Poster Session 2**

Wednesday, April 14

11:00 AM – 12:00 PM **Development of human resident immunity in mucosal sites**
Dr. Donna Farber

12:00 PM – 1:00 PM Break

1:00 PM – 2:30 PM **Session: Human Immunology**
Chairs: Drs. Thomas Murooka & Nathan Peters

1:00 pm Effect of hookworm infection and anthelmintic treatment on naturally acquired antibody responses against the GMZ2 malaria vaccine candidate and constituent antigens
Benjamin Amoani, bamoani@ucc.edu.gh

1:15pm Early reduction in PD-L1 expression predicts faster treatment response in human cutaneous leishmaniasis
Nidhi Sharma Dey, nidhi.dey@york.ac.uk

1:30pm Adoptive transfer of helminth antigen stimulated human PBMCs attenuates disease progression in a humanised mouse model of graft-versus-host disease
Sandra O'Neill, sandra.oneill@dcu.ie

1:45pm CD8+CD57+T cells in the pathogenesis of disseminated leishmaniasis
Thiago Cardoso, thiago.cardoso@fiocruz.br or tmarconicardoso@gmail.com

2:00pm Interleukin 17 stimulates mononuclear cells to kill Echinococcus granulosus by NO-dependent mechanism: immuomodulation by laminated layer
Manel Amri, manelamri@yahoo.fr

2:15 PM – 2:45 PM Break

2:45 PM – 4:15 PM **Session: Mucosal and barrier tissue immunology**
Chairs: Drs. Pedro Gazzinelli & Tiffany Weinkopff

2:45pm Helminths battle type 2 immunity for control of the intestinal stem cell niche
Danielle Karo-Atar, danielle.atarkaro@mail.mcgill.ca

3:00pm Deletion of Matrix Metalloproteinase 17 renders Mice resistant to chronic Trichuris muris Infection by affecting epithelial Goblet Cell-specific Immune-effectors
Pia Vornewald, pia.vornewald@ntnu.no

3:15pm Cysteinyl leukotriene receptor-1 is required for clearance of Nippostrongylus brasiliensis and development of protective memory responses during secondary infection
Paballo Mosala, pablovo92@gmail.com

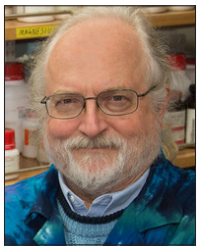
3:30pm IL-11 regulates innate mucosal immunity in acute helminth infection
Jonah Kupritz, kupritzjl@nih.gov

3:45pm Basophils promote tissue-resident optimal Th2 cell function during helminth infection
Lauren Webb, lwebb2@uw.edu

4:00pm Cellular dynamics of immune evasion during Leishmania major infection
Romaniya Zayats, umzayatr@myumanitoba.ca

4:15 PM – 5:00 PM **Closing remarks and awards**

KEYNOTE SPEAKERS



Modulation of host responses and virulence by protozoan RNA viruses

Dr. Steve Beverley (Washington University)

Many eukaryotic microbes possess RNA virus-like elements, the role and significance of which are usually unknown. We have studied the trypanosomatid protozoan parasite *Leishmania* in South America, which often bear the dsRNA virus LRV1, as a new paradigm of protozoal viral virulence. Like most Totiviruses, LRV1 is neither shed nor infectious, and thus may be viewed as a persistent endobiont. Perspectives on the importance of protozoal viruses changed upon discovery that *L. guyanensis* LRV1 is associated with hypervirulence and increased metastasis in animal models, the latter being a hallmark of the more severe forms of leishmaniasis (Ives *et al. Science* 2011). For *Leishmania* we developed RNA interference or antiviral tools for reproducibly generating isogenic lines lacking LRV1s (Brettmann et al PNAS 2016; Kuhlmann *et al PNAS* 2017). This has allowed extension of findings with *L. guyanensis* to *L. braziliensis*, the predominant agent of mucocutaneous leishmaniasis (MCL). In murine infections Leishmania bearing LRV1 display many alterations in host responses as shown by the work of Nicolas Fasel's group, perhaps most significant being the induction of Type-I interferons (*Rossi et al PNAS* 2017). How these combine to promote LRV1-mediated hypervirulence is a key question.

One question is the contribution of LRV1 with *Leishmania* pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models, Many but not all studies have reported an association of LRV1 with more severe forms of leishmaniasis (Cantanhêde *et al PLoS NTD* 2015), and LRV1 was associated with increased relapse and/or treatment failures in human *L. braziliensis*-infected patients treated with pentavalent antimonials in Peru and Bolivia, as well as in *L. guyanensis* infections treated with pentamidine (Adauai *et al* & Bourreau *et al. J. Inf. Dis* 2016). The association of LRV1 with clinical drug treatment failure could serve to guide more effective treatments through the use of LRV1 inhibitors.

Currently we have embarked on a systematic survey of known and new viruses in *Leishmania* as well as their monoxenous insect trypanosomatid relatives and other parasites including the Apicomplexans *Toxoplasma* and *Plasmodium*, using a wide range of methods including next-gen RNA sequencing (Grybchuk *et al PNAS* 2018). This has greatly expanded our knowledge of the parasitic protozoal virome with the discovery of multiple new viruses, several of which may play similar roles impacting virulence through different mechanisms. The properties, evolution and potential contributions of these to virulence and biology will be discussed.

Memory B cell responses to infection

Dr. Marion Pepper (University of Washington)

Dr. Marion Pepper earned her Ph.D. in Immunology in 2006 from the University of Pennsylvania School of Medicine. Her work there focused on the development of the CD4+ T cell response to the eukaryotic parasite, Toxoplasma gondii in the laboratory of Dr. Christopher A. Hunter. She continued to study the adaptive immune response, specifically focusing on memory lymphocyte differentiation and function in Dr. Marc K. Jenkins' lab at the University of Minnesota. In 2011, she joined the faculty of the Department of Immunology at the University of Washington in Seattle as an Assistant Professor. The overarching goals of her laboratory are to understand how to regulate immune cell differentiation such that memory responses against infections can be optimized, while those against allergy can be suppressed. Her studies have revealed key differentiation programs and functions of both memory CD4+ T cells and B cells in response to pathogens and allergens. In 2017, she was awarded the Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Diseases award and was promoted to Associate Professor. She enjoys living in Seattle with her husband, two daughters, one very large dog and a recently acquired puppy.

Development of human resident immunity in mucosal sites

Dr. Donna Farber (Columbia University)

Donna L. Farber, Ph.D. is the George H Humphreys, II Professor of Surgical Sciences (in Surgery) and Professor of Microbiology and Immunology at Columbia University. The focus of Dr. Farber's research is on adaptive immunity, particularly to virus infections and how T cell differentiate and generate long-term immunological memory in diverse tissues sites. Dr. Farber's laboratory identified subsets of tissue-resident memory T cells in the lung that mediate protective immunity to respiratory virus infection and has led an initiative in translational immunology to dissect human immune responses in tissues throughout the body, in multiple mucosal and lymphoid tissues from individual organ donors of all ages. She has also made important contributions to understanding how T cells seed tissues and mediate responses to viruses during different life stages including infancy. Dr. Farber leads NIH/NIAID-funded Program grants on human immunity, anti-viral responses and is part of the Human Immunology Project Consortium (HIPC), as well as the NIH/NHLBI consortium on human lung aging. In addition to the NIH, her research is supported by the Helmsley Charitable trust and the Chan-Zuckerberg seed network for the Human Cell Atlas. She has over 140 publications and has served on numerous advisory committees for the NIH, American Association of Immunologists, Federation of Clinical Immunology Societies and others.

ABSTRACTS

Session: Neuroimmunology

Enteric helminth coinfection enhances host susceptibility to neurotropic flaviviruses via a tuft cell-IL-4 receptor signaling axis

Pritesh Desai (Washington University in St. Louis)

Although enteric helminth infections modulate immunity to mucosal pathogens, their effects on systemic microbes remain less established. Here, we observe increased mortality in mice coinfected with the enteric helminth Heligmosomoides polygyrus bakeri (Hpb) and West Nile virus (WNV). This enhanced susceptibility is associated with altered gut morphology and transit, translocation of commensal bacteria, impaired WNV-specific T cell responses, and increased virus infection in the gastrointestinal tract and central nervous system. These outcomes were due to type 2 immune skewing, as coinfection in Stat6-/- mice rescues mortality, treatment of helminth-free WNV-infected mice with IL-4 mirrors coinfection, and IL-4 receptor signaling in intestinal epithelial cells mediates the susceptibility phenotypes. Moreover, tuft cell-deficient mice show improved outcomes with coinfection, whereas treatment of helminth-free mice with tuft cell-derived cytokine IL-25 or ligand succinate worsens WNV disease. Thus, helminth activation of tuft cell-IL-4-receptor circuits in the gut exacerbates infection and disease of a neurotropic flavivirus.

P. berghei Infection Expands Microglia Populations Associated with Activation and Proliferation and Induces Upregulation in Inflammation Related Genes

Marshall Roedel (University of Utah)

Cerebral malaria is a complication of Plasmodium falciparum infection which most frequently affects young children less than 5 years of age. It can result in seizures, intracerebral hemorrhage, and is often fatal. These symptoms are the effect of the disruption of the blood brain barrier in response to the localization of Plasmodium-infected red blood cells in the neural vasculature. The blood brain barrier is a complex of cells that tightly regulate the exchange of molecules between the brain and the blood. It consists of endothelial cells that form the vessel walls and that are connected by densities of proteins called tight junctions, an extracellular protein scaffolding, and various cell types that interact with the endothelial cells. Microglia, a heterogenous population of macrophage-like cells in the brain have been shown to be important regulators of the blood brain barrier during malaria infection. They have been shown to phagocytize infected red blood vessels, and in culture are activated by extracellular vesicles derived from infected red blood cells. Sequencing of bulk microglia RNA in a mouse model of cerebral malaria have confirmed that expression of genes related to activation and immune response is altered in infected mice compared to controls. However, this approach is unable to differentiate responses between different subpopulations of microglia. Here, we have used single cell RNA sequencing to observe different subpopulations of microglial cells that respond differently during experimental cerebral malaria in C57BL/6J mice. Our analysis yielded eight clusters associated with homeostasis, activation, and proliferation, as well as a population that expressed markers for perivascular macrophages. Differential expression between naïve and infected groups yielded an upregulation in genes associated with antigen presentation, cytokine expression, and interferon response in infected mice.

Investigating astrocyte subsets during Toxoplasma gondii infection

Zoe Figueroa (University of California, Riverside)

Toxoplasma gondii infects a third of the world's population and is asymptomatic except during times of immunocompromise when infection leads to severe neurological damage. The immune response in the brain is characterized by "reactive astrocytes". Astrocytes are known to help physical and metabolic support for neurons, axon guidance, synaptic support and control of the blood brain barrier. When injury, disease, or infection occur in the CNS, astrogliosis occurs and astrocytes become reactive. RAs are defined by their increased proliferation, enlarged cell bodies and processes and change in function. Yet it is unclear if RAs contribute to or help alleviate disease progression, but overall astrocyte reactivation has been discovered to be an important contributor to several neurological diseases. During Toxoplasma infection IFN-ɣ-dependent responses of astrocytes are required to mount a protective immune response in the brain. During infection, astrocytes can inhibit parasite replication via IFN-ɣ-mediated activation of STAT1, demonstrating a neuroprotective function. Contrarily, astrocytes are responsible for pathological changes in neurochemistry in the infected brain following downregulation

of glutamate transporter 1 (GLT-1). Characterization of these cells, using a new reporter mouse where Cre is driven by the LCN2 promoter, we can specifically target reactive astrocytes, a key step in determining RA heterogeneity. Using flow cytometry, we have characterized surface protein subsets found during chronic Toxoplasma infection. Experiments demonstrate that during infection, a potential chronic reactive astrocyte subset (cRA) is present. To determine if these astrocytes define a chronic inflammatory state, 10X experiments on bulk astrocytes at various stages of infection are being conducted to determine if similar populations are expressed during acute compared to chronic infection as well as during other chronic stimulants. Furthermore, our Lcn2CreERT2;TdTomato reporter mouse will for the first time allow identification of genes uniquely altered in RAs during Toxoplasma providing a novel tool for future study.

Helminth-activated monocytes inhibit microglial activation and neuroinflammation

Jianya Peng (Rutgers, Center for Immunity and Inflammaiton)

It is well established that the recruitment of peripheral cells into the central nervous system can promote neurodegenerative disorders. However, emerging studies suggest that specialized immune cells can also perform neuroprotective functions. Given the need for new therapies to treat neurologic deficits, gaining a better understanding of these cellular phenotypes is of critical importance. Helminth infections have greatly informed our knowledge of immune cell plasticity; however, the ability of helminth-activated cells to modify neuroinflammation remains poorly defined. Here we report that Trichinella spiralis infection promotes type 2 cytokine-independent monocyte responses in the brain that are required to inhibit excessive microglial activation and host death. Brain-protective monocytes express immunoregulatory molecules that are sufficient to inhibit TNF production from activated microglia. Importantly, Trichinella-infected mice are also protected from subsequent LPS-induced neuroinflammation. These studies identify a functionally distinct population of monocyte with neuroprotective qualities and demonstrate that helminths can modulate neuroinflammation.

Lymphatic drainage of cerebrospinal fluid regulates peripheral T cell responses during Toxoplasma gondii brain infection

Michael Kovacs (University of Virginia)

Toxoplasma gondii is an intracellular protozoan parasite that causes chronic brain infection in approximately one-third of the world's population. Mouse studies have demonstrated that continual recruitment of circulating T cells to the brain is necessary for parasite control. Nonetheless, it has remained unclear how peripheral T cell responses against the parasite are maintained as the brain is devoid of lymphatic vessels, which in other tissues transport antigen and antigen-presenting cells to lymph nodes for T cell activation. Recently, it was discovered that the dura mater layer of meninges harbors a previously unrecognized network of lymphatic vessels that drain cerebrospinal fluid (CSF)-borne protein and immune cells. Here, we test the hypothesis that lymphatic drainage of CSF promotes peripheral T cell responses during T. gondii brain infection. In support of this hypothesis, we find that T cell activation increases significantly in the deep cervical lymph nodes (DCLNs) following progression to the chronic phase of infection in the brain. Moreover, blockade of CSF outflow via lymphatic vessel ligation leads to a reduction in T cell responses in the DCLNs, as measured by classical T cell activation markers and IFNγ-gamma production. Studies using DQ-OVA, which tracks antigen capture and processing, reveal that CSF protein is sampled in the DCLNs as well as the dura mater, where the CSF-draining lymphatic vessels originate. The presence of Nur77+ antigen-activated T cells at these sites suggests possible uptake and presentation of antigen locally. Finally, we report a reduction in the rate of CSF lymphatic drainage during T. gondii brain infection relative to baseline, the biological consequences of which remain uncertain. To conclude, we demonstrate that peripheral T cell responses against T. gondii are supported by lymphatic drainage of CSF to the DCLNs, with local sampling of antigen in the dura mater potentially contributing to peripheral T cell activation.

Tuft cell-derived acetylcholine regulates epithelial fluid and mucus secretion in Type 2 immunity

Tyler Billipp (University of Washington)

The intestinal epithelium maintains a barrier against microbiota, pathogens, and environmental insults in part through the secretion of fluid and mucus. During the Type 2 immune response to parasitic helminths, IL-13 signaling drives dramatic epithelial remodeling, resulting in goblet cell hyperplasia and increased mucus production that contribute to the "weep and sweep" required for helminth clearance. Tuft cells, rare chemosensory epithelial cells that initiate this Type 2 response upon sensing of helminths or certain microbial metabolites, also express the enzyme ChAT required for synthesis of acetylcholine (ACh). ACh is a potent inducer of epithelial fluid and mucus secretion but is typically thought to be produced by enteric neurons. We find that during homeostasis the microbial metabolite

succinate induces rapid fluid and mucus secretion in the distal small intestine dependent on tuft cell-derived ACh. Secretion also requires tuft cell chemosensing and is independent of neuronal involvement. Fluid and mucus secretion are enhanced during Type 2 inflammation, consistent with the observed increase in ChAT+ tuft cells. Upon infection with the hookworm Nippostrongylus brasiliensis, tuft-specific ChAT-deficient mice suffer delayed worm clearance despite robust epithelial remodeling. Our findings suggest that upon sensing of luminal signals produced by helminths and microbes tuft cells stimulate an epithelium-intrinsic effector unit composed of enterocytes and goblet cells to rapidly respond with fluid and mucus secretion. This response is amplified by the epithelial remodeling that occurs during the Type 2 response, contributing to anti-helminth immunity.

Beta-amyloid's antimicrobial properties protect elderly mice from Toxoplasma gondii infection in the face of age-related immunological decline.

Kathryn McGovern (University of Arizona)

Data suggest that beta-amyloid (Aβ) is a major initiator of Alzheimer's disease (AD) and a highly conserved anti-microbial peptide. Although Aβ accumulation is thought to be detrimental to the brain as a trigger of pathology, like neuroinflammation, efforts to cure AD by clearing Aβ have failed in part because the reactivation of latent disease. Thus, more work is needed to understand how Aβ may be necessary to the brain, where the actions of the adaptive immune system are more restricted compared to other organs in the body. Using the intracellular T.gondii, we are exploring how infection impacts Aβ levels in the brain, how Aβ may combat the parasite, and whether Aβ's importance may change as organisms age. We found that exposure to Aβ is effective at protecting cells from invasion by the parasite and those parasites replicate more slowly within cells once they do invade. In vivo, mice with excess Aβ have a lower parasite burden in the brain, indicating that Aβ may either help protect the brain from colonization by an invading pathogen or can help clear the brain of pathogens that have breached the blood-brain barrier. Further, we have found that these protective effects are only beneficial to elderly mice as aged (18mo+) mice with excess Aβ are able to survive T.gondii infection, while their WT littermates are not. These data support the idea that the Aβ could be beneficial as we age. Current work is focused on whether Aβ is toxic to T.gondii or if, as seen in viruses, Aβ traps the pathogen, making it more vulnerable to clearance by the immune system. Future work will include examining how Aβ impacts the functions of the immune system and how pathogens like T.gondii may have evolved ways to subvert the antimicrobial actions of Aβ.

Session: B cells and humoral immunity

T follicular helper (Tfh)-germinal centre (GC) B cell response is required for sterile immunity during enteric helminth infection

Aidil Zaini (Monash Biomedicine Discovery Institute, Clayton, Australia)

CD4 T follicular helper (Tfh) cells are an important component of germinal centres (GCs), whose overarching goal is to produce an effective humoral immunity. Yet, the significance of Tfh-GC responses during helminth infections is controversial and poorly understood. Herein we report that a diminished Tfh-GC B cell response is associated with chronic infection of the helminth parasite Trichuris muris (Tm). We show that a high-dose (HD) of parasite eggs in mice that leads to a Th2 cell-biased acute infection and worm clearance, results in a significant increase in Tfh and GC B cells, which is typified by the selection of parasite-specific IgG1 class switching. In contrast, a low-dose (LD) infection that results in a Th1 cell-biased chronic infection fails to induce a potent Tfh-GC response. Using an IL-4/IL-21 dual reporter mouse, we further demonstrate that IL-4-producing Th2 cell response in the mesenteric lymph nodes predominantly occurs during the first stages of HD infection while a potent Tfh-GC response is prominent during the later stages of worm expulsion. Additionally, the loss of B cell-intrinsic IL-4Rα signals results in a delay in worm expulsion. Blockade of Tfh-GC interactions during HD infection promotes chronic infection, suggesting Tfh and GC B cells are required for complete worm clearance and are thus required for sterile immunity. Collectively, our data provide insights into the roles of Tfh-GC response during helminth infection and identify a potent Tfh-GC response as a protective component of type 2 immunity to intestinal helminths.

Characterization of intercellular communication between B cells exposed to Leishmania donovani

Tanja Stögerer (Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique)

Polyclonal B cell activation and resulting hypergammaglobulinemia are a detrimental consequence of visceral leishmaniasis, which can be caused by the protozoan parasite Leishmania donovani; however, the mechanisms underlying this excessive production of non-protective antibodies are still poorly understood.

While studying the interaction of B cells with L. donovani in search of the mechanism underlying polyclonal B cell activation, we observed the B cells to form long tubular protrusions when exposed to the parasite. We have characterized these protrusions to be primarily actin-based with lower content of tubulin, which is in line with literature descriptions of tunneling nanotubules (TNTs). These TNTs represent a novel way of intercellular communication via the passage of material along these formed connections, which has been implicated in the spread of some pathogens. Interestingly, we have observed parasites to be situated on these intercellular protrusions between splenic B cells exposed to L. donovani, suggesting that amastigotes may be gliding from one cell to another using TNTs. To elucidate a possible role of these connections in the propagation of parasite cell activation, we studied whether activation could be passed on through soluble messengers such as cytokines and found this to be insufficient to pass on the activation state between B cells. On the other hand, preliminary experiments allowing for cell-to-cell contact suggest that parasites and the activation state can be passed from cell to cell.

Taken together, we provide novel insights about TNT formation in primary splenic B cells and the possibility that this mechanism may contribute to polyclonal B cell activation.

Secondary antigen stimulation contributes to the heterogeneity of pathogen-specific CD8+ T cell response during acute and chronic toxoplasmosis

Lindsey Shallberg (University of Pennsylvania)

Many factors have been described as important in the development of T cell heterogeneity, particularly those that influence fate decisions. These include cytokines such as IL-2 and IL-15, as well as transcription factors such as eomesodermin and T-bet, yet it is less clear whether secondary antigen encounter and TCR activation alters CD8+ T cell fate decisions. To address this issue, OT-I T cells, specific for the SIINFEKL peptide from OVA, which express the Nur77-GFP reporter of recent TCR activation, were paired with transgenic T. gondii that express OVA to follow the evolution of parasite-specific CD8+ T cell responses at different stages of this chronic infection. In vivo studies revealed that Nur77-GFP was a faithful reporter of CD8+ T cell recognition of infected cells and the kinetics and levels of TCR stimulation in infected tissues correlated with parasite burden. Adoptive transfer of Nur77-GFP negative and positive OT-I from infected mice into naive recipients resulted in formation of similar memory populations. However, in the brain, Nur77-GFP+ OT-I were transcriptionally distinct from Nur77-GFP- cells and recent antigen exposure was associated with distinct groups: highly differentiated effectors, IFNγ producers, and a population of CD103+ CD69+ Trm like cells. The use of therapies or mutant parasites revealed that during chronic toxoplasmosis that the presence of persistent antigen and associated TCR signals contribute to the T cell heterogeneity observed.

Dissecting the role of helminth antigen exposure in reprograming hematopoietic development

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 helminth infection alters the immune responses of offspring is lacking but is critical to improve childhood vaccine regimens in endemic areas, 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, similar to what has been reported in humans, measured by vaccine titer. Additionally, these pups have reduced humoral immunity cell frequencies in the draining lymph node following Tetanus/Diphtheria immunization. To determine the origin of this humoral immunity defect, we began investigating lymphoid progenitors. We found an increase in the common lymphoid progenitors (CLPs) in the bone marrow of naïve mice from infected mothers. Additionally, there is an increase in B cell skewing CLPs in pups from infected mothers, but a decrease in transitional B cells leaving the bone marrow. When immunized with a Tetanus/Diphtheria vaccination, there is a significant reduction in expansion of these progenitors in comparison to age matched controls from uninfected mothers coupled with a decrease in bone marrow B cells. RNA sequencing revealed a defect in stem cell pluripotency signaling pathway, including the differential expression of ID2 and TCF3, which both impact B cell differentiation and maturation. Further analysis post-immunization shows a decrease in immature B cells in the bone marrow of pups from infected mothers, suggesting a more exclusive selection process or differential selective pressure

than in pups from uninfected mothers, leading to lower B cell frequencies in the draining lymph node. We hypothesize that altered transcriptional regulation at the progenitor level caused by maternal Schistosomiasis is the mechanistic root of long-lived defects in humoral immunity to foreign antigens.

An invariant Trypanosoma vivax vaccine antigen inducing protective immunity

Gavin Wright, Delphine Autheman (Wellcome Sanger Institute and University of York)

Animal African Trypanosomiasis (AAT) has a significant impact on animal agriculture in Sub-Saharan Africa by threatening the livelihood of farmers and food security in endemic countries, and is mainly caused by two species of African trypanosomes, Trypanosoma congolense and T. vivax. Such is the impact of this disease that the Food and Agricultural Organisation of the United Nations consider this disease to “”lie at the heart of African poverty””. While vaccination would be an ideal solution to manage AAT, effective subunit vaccines against African trypanosomes have not yet been identified because of the evolution of sophisticated immunoprotective strategies such as antigenic variation.

We have used a reverse vaccinology approach to identify antigenically invariant proteins on the surface of T. vivax parasites to be used as vaccine targets. We assembled a library of forty proteins predicted to be associated with the surface of the parasite bloodstream-forms by expressing the entire extracellular regions as secreted recombinant proteins in mammalian cells to increase the chance that vaccine-elicited antibodies are raised against native parasite epitopes. We systematically vaccinated mice with purified proteins and challenged them with T. vivax parasites to evaluate the proteins’ efficacy as vaccine candidates. We identified a non-variant antigen (V23) that can elicit sterile protection and demonstrated its localisation to the parasite flagellum. Naïve mice were also protected by adoptive transfers of either V23 immune serum or recombinant monoclonal antibodies directed against V23. By individually mutating antibody effector binding sites on protective recombinant antibodies, complement recruitment was found to be a major protective mechanism. Our results identify this antigen as a leading vaccine candidate against AAT.

Session: Immune regulation, the microbiota, and host and parasite metabolism

Staphylococcus aureus induces Trichuris muris egg hatching via a contact dependent mechanism

Amicha Robertson (Vilcek Institute at NYU Langone Health)

The life cycle of the murine intestinal parasite, Trichuris muris, begins with an egg hatching step in the caecum. Gram-negative members of the gut microbiota trigger this hatching process by attaching to the egg via fimbriae. However, previous studies and our data show that the non-fimbriated Gram-positive bacterial species, Staphylococcus aureus, can also trigger egg hatching in vitro through an unknown mechanism. Given the increasing evidence implicating S. aureus as an important variable within the human gut microbiota community, especially during childhood development, we used an in vitro hatching assay to investigate how S. aureus affects this critical stage of the helminth life cycle. We show that eggs exposed to higher concentrations of bacteria hatch at a faster rate and that live bacterial cells need to be present in the mixture for hatching to occur. Scanning electron microscopy experiments show that S. aureus closely associates with the polar region of the egg, and we show that this physical contact is necessary for hatching to take place. Lastly, we show that active protein synthesis in S. aureus is important for hatching. These findings provide insight into a transkingdom interaction that might influence the development of intestinal helminth infections.

Small intestinal levels of the short-chain fatty acid isovalerate are elevated during Heligmosomoides polygyrus infection and can promote helminth fecundity

Mia Kennedy (Department of Biochemistry and Microbiology, University of Victoria)

Heligmosomoides polygyrus is a natural intestinal helminth parasite of mice, which is widely used as a model of chronic small intestinal helminth infection. While it is known that infection with H. polygyrus alters the composition of the host’s bacterial microbiota, the functional implications of this alteration are unclear. We investigated the impact of H. polygyrus infection on short-chain fatty acid (SCFA) levels in the mouse intestine and sera. We found that helminth infection resulted in significantly upregulated levels of the branched SCFA isovaleric acid, exclusively in the proximal small intestine, which is the site of H. polygyrus colonization. We next set out to test the hypothesis that elevating local levels of isovaleric acid was a strategy used by H. polygyrus to promote its own survival. To test this, we

supplemented the drinking water of mice with isovalerate during H. polygyrus infection and examined whether this improved helminth fecundity or chronicity. We did not find that isovaleric acid supplementation affected chronicity, however, we found that it did promote helminth fecundity, as measured by helminth egg output in the feces of mice. Through antibiotic-treatment of helminth-infected mice, we found that the bacterial microbiota were required in order to support elevated levels of isovaleric acid in the small intestine during helminth infection. Overall, our data suggests that during H. polygyrus infection there is a microbiota-dependent increase in the local, small intestinal production of isovaleric acid, and this supports helminth fecundity in the murine host.

Immunological dynamics of gastrointestinal parasitism: investigating the relationship between Trichuris muris trickle infection, the mucosal barrier, and the microbiota

Stefano Colombo (Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, United Kingdom)

The kinetics of gastrointestinal (GI) helminth burden in human populations – in regions where these parasites are endemic – show a dynamic profile, rapidly increasing in childhood, markedly falling in adolescence, then plateauing through adulthood. This indicates a partially protective immune response develops over time, transitioning from one ineffective at resisting infection, to one primed to expel helminths, then settling into a homeostatic balance limiting worm burden but not providing concomitant immunity. Traditional laboratory murine single-dose infections fail to model this complex relationship between the host immune response and the parasite, typically reflecting either absolute resistance or susceptibility to infection. To address this, we developed a model Trichuris muris infection system where small doses of infective T. muris eggs are regularly administered (trickle infection). We observed that T. muris trickle infection drove infection kinetics comparable to those seen in human and wild rodent populations in endemic regions. Further, we observed dynamic phenotypic changes in the T-helper (Th) immune response and the mucosal barrier coincident with the kinetics of the infection. T. muris trickle initially drove a strong IFNɣ-dominated Th1 response. However, as worm burden rose the Th balance shifted to Th2-dominated and protective barrier mechanisms such as goblet cell hyperplasia were established. This coincided with increased resistance to subsequent infections, and long-term protective immunity. The host microbiota is a primary mediator of mucosal immune responses, however, its role in helminth infection is poorly understood. We performed longitudinal 16S metagenomic sequencing in our model. We observed complex, dynamic changes in the host microbiota correlated with the immune response not previously observed in single-dose helminth models. Our data demonstrates a relationship between the host microbiota and the mucosal immune phenotype. Thus, T. muris trickle infection presents a unique model to investigate mechanisms that regulate dynamic changes in immunological and physiological responses to GI helminths.

Control of systemic eosinophilia by a protozoan commensal dictates asthma severity.

Arthur Mortha (University of Toronto)

The local composition of immune cells within any given organ determines its susceptibility to infections and autoimmunity. The intestinal microbiome, a collection of bacteria, viruses, fungi and commensal protozoa, shapes the phenotype, function and abundance of adaptive and innate immune cells inside and outside of the intestinal tract across multiple gut-tissue axes. During asthma, group 2 innate lymphoid cells (ILC2), T helper (h) cells, B cells and eosinophils show marked alterations in function and abundance, collectively promoting the pathology of this disease.

Here, we report for the first time, that the murine protozoan commensal Tritrichomonas musculis (T.mu) imprints a lung-specific immune landscape after colonizing the intestinal tract. Following permanent engraftment as a new member of the gut microbiota, mice carrying T.mu did not develop spontaneous asthma, despite showing significantly elevated levels of lung eosinophils. This adaptation in the local lung immune landscape was driven by a tripartite interaction of gut-derived, migratory ILC2s, and lung-resident Th cells and B cells. Mechanistically, locally activated, gut-resident ILC2s, migrated to the lung to promote eosinophil accumulation through an ICOS/ICOSL-dependent pathway. Strikingly, ILC2s were not sufficient to facilitate local eosinophilia in the lung and required interactions between Th cells and B cells to facilitate the protozoan-driven immune adaptation. Lastly, the permanent remodeling of the lung immune landscape following protozoan colonization, exacerbated the house dust mite-induced allergic airway 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.

Mechanisms of gut microbiota mediated expansion of granulocyte monocyte progenitors

Brandon Thompson (University of Virginia)

Bile acids are an important class of host-derived, microbially metabolized, metabolites. Primary bile acids are created from cholesterol breakdown in the liver and are transported to the gut where they aid in the emulsification of fats, lipids, and fat-soluble vitamins. Those that are not metabolically consumed travel through the gut until they are further metabolized by the gut microbiota into secondary bile acids. Recent literature has suggested that bile acids can, in addition to aiding in metabolism, also act as potent immune signaling molecules. Indeed, we have shown that elevated serum concentrations of secondary bile acid, deoxycholic acid (DCA), is associated with protection from infection with amebic parasite Entamoeba histolytica in both humans as well as a mouse model of amebic infection. Protection from infection was associated with an expansion of bone marrow resident immune cell progenitors, granulocyte monocyte progenitors (GMPs). GMPs give rise to mature circulating immune cells, including neutrophils and monocytes. To determine if DCA was directly interacting with the bone marrow environment we performed colony-forming assays, utilizing whole marrow, in the presence or absence of DCA. We observed that cells that were allowed to grow in the presence of DCA formed significantly more GMP-associated colony forming units compared to media controls. Importantly, this phenotype did not occur when treating with primary bile acid, and DCA precursor, cholic acid, suggesting that the expansion of GMPs is DCA specific. Finally, we observed that DCA mediated expansion of GMPs was dependent upon the vitamin D receptor (VDR) as VDR-/- cells treated with DCA did not show an expansion of GMPs when compared to littermate controls. These data suggest that the gut microbiome, via bile acid metabolism, can profoundly alter the bone marrow environment, leading to fundamental changes to the immune system, increasing GMPs, and resulting in protection from amebic infection.

Amino acid transport acts as a rheostat of ILC2 responses

Suzanne H Hodge (University of Manchester)

Innate lymphoid cells (ILCs) are transcriptionally poised and pre-primed cells, enriched at barrier sites including the lung, skin and intestinal tract. ILCs respond rapidly to danger signals, and are integral for maintaining homeostasis and providing host immunity in response to helminth infections. In particular, group 2 ILCs release the type 2 cytokines IL-5 and IL-13 to mediate protective immunity, yet have also been implicated in driving inflammation in allergic diseases. Therefore, there is an urgent need for a better understanding of the cell-intrinsic mechanisms which facilitate rapid type 2 innate responses in the tissue microenvironment, and the checkpoints that determine appropriate ILC functions. Recent evidence have implicated changes in cell-intrinsic metabolism as a critical regulator of ILC function. Moreover, it is increasingly understood that lymphocyte sensing of nutrient availability in the tissue environment is required to engage cellular metabolism and to generate the energy and biomass required to fuel immune effector function. In particular, fundamental metabolic substrates – such as essential amino acids – act to fuel biosynthesis and provide environmental cues that provide a rheostat for cellular metabolic function. We have identified that amino acid availability is altered during helminth infection and that ILC2 are preferentially poised to import essential amino acids. The uptake of amino acids by ILC2 acted to modulate the activation of the master metabolic sensor, mTOR, and to license proliferation. Moreover, we identify amino acid transporters preferentially expressed by ILC2 and utilizing conditional deletion of amino acid transporters we are dissecting the role of amino acid transport in determining ILC effector function at homeostasis and in the context of infection or inflammation. Together our data suggest nutrient sensing and amino acid import by ILC2 act as critical regulators of type 2 immune responses. These findings may help to inform novel therapeutic strategies aimed at targeting ILC2 to boost protective immunity to parasitic infections or to suppress excessive responses in the context of allergic disease.

Breathing is Eating: A Toxoplasma Oxygen Sensor Mediates Virulence By Regulating Tryptophan Scavenging in Response to IFNγ

Charlotte Cordonnier (University at Buffalo)

Toxoplasma gondii is an obligate intracellular parasite that is an important opportunistic infection in AIDS patients and other immunocompromised individuals. Human infection typically occurs through the ingestion of contaminated food or water. After breaching the intestinal epithelial barrier, the parasite spreads to a large variety of other organs such as the brain, eyes and placenta. During its journey through a host, the parasite must successfully adapt to its environment conditions and hijack host cellular proteins and nutrients in order to proliferate. Oxygen (O2) is one such environmental factor. Cytoplasmic prolyl 4-hydroxylases (PHDs) are O2 sensors that regulate cellular responses to changes in O2 availability. Toxoplasma expresses two PHDs. One of them, TgPHYa hydroxylates Skp1, a subunit of the E3-SCF ubiquitin ligase complex. In vitro, TgPHYa is important for growth at low O2 levels. However, no studies have focused yet on the in vivo importance of TgPHYa. Using a type II ME49 Toxoplasma TgPHYa knockout, we report that TgPHYa is important for Toxoplasma

virulence and brain cyst formation. We further find that while TgPHYa mutant parasites can establish an infection in the gut they are unable to efficiently disseminate to peripheral tissues because the mutant parasites are unable to survive within inflammatory monocytes. Since this phenotype abrogated in IFNɣ knockout mice, we studied how TgPHYa mediates survival in IFNɣ-treated cells. We find that TgPHYa is not required for deployment into the host cell of parasite-encoded effectors that neutralize anti-parasitic processes induced by IFNɣ. In contrast, we find that TgPHYa is required for the parasite to scavenge tryptophan, which is an amino acid whose levels are decreased after IFNɣ upregulates the tryptophan-catabolizing enzyme, indoleamine dioxygenase. Together, these data reveal that parasite regulation of nutrient scavenging is novel mechanism to avoid IFNɣ-mediated killing.

Session: Technology, informatics, and unique models of disease

Understanding granuloma heterogeneity in experimental visceral leishmaniasis through spatially-resolved multi-omic approaches.

Shoumit Dey (Hull York Medical School, University of York)

The leishmaniases are a group of neglected tropical diseases with diverse clinical presentations. As found in other infectious (e.g. TB, schistosomiasis) and non-infectious diseases (arthritis, cancer), most forms of leishmaniasis are characterised histo-pathologically by granulomatous inflammation. In experimental visceral leishmaniasis, hepatic granulomas serve as focused inflammatory microenvironments with at a core of parasite-infected Kupffer cells surrounded by a loosely-organised immune cell infiltrate. Early during the process of granulomatous inflammation, infected Kupffer cells activate uninfected bystander cells, a process thought to limit excess pathology. Computational modelling of individual granulomas has suggested a degree of heterogeneity with some rapidly resolving parasite load and others supporting parasite recrudescence. To further understand the mechanisms and consequences of granuloma heterogeneity, we have adopted a spatially resolved multi-omic approach, based on highly-multiplexed protein and mRNA profiling (NanoString GeoMx; focusing on immune cell subset composition, activation status and checkpoint pathways) and mass spectroscopy imaging (MSI; focusing on lipidomics). Applying graph-based clustering of immune protein expression data to initially explore granuloma heterogeneity, we have identified two granuloma sub-types. These subtypes can be identified independent of granuloma size and reflect differences in composition and activation status, as measured by CD11c, F4/80, MHCII and GZMB expression. Of note, granulomas associated with a lower activation profile shows a significantly elevated CD19 expression suggesting a role for B cells in modifying the granuloma microenvironment. Further studies using MSI are ongoing to determine whether granuloma sub-types identified in this way relate to differences in the underlying metabolic profile. This analysis will provide novel insights into granuloma heterogeneity that will both inform our understanding of the pathogenesis of visceral leishmaniasis and more generally provide a greater understanding of the interface between inflammation and metabolism in granulomatous diseases.

Using re-wilded mice to characterize the inferential power of ecoimmunological assays for describing immune phenotype

Alexander Downie (Department of Ecology and Evolutionary Biology)

Comparative studies of immune defense in non-model organisms have tremendous potential for shedding light on immune strategies and the ecological factors shaping them. However, researchers designing such studies face a number of logistical and methodological challenges. One particularly thorny issue confronting immunologists and disease ecologists is the dearth of available reagents for the in-depth molecular study so common in human and mouse immunology. This prevents researchers from describing immune phenotype in any detail. Here, we confront this problem by exploring the ability of simple immune assays that can be conducted in any vertebrate species to serve as proxies for more detailed aspects of immune defense that can only be measured with specialized reagents in lab settings. We build on our previously-published results describing the immune responses of re-wilded laboratory mice that are kept in outdoor enclosures and allowed experience of natural microbial environments. With samples from these mice, we examine whether antibody concentrations can serve as predictors for immune cell and microbiome composition, as well as cytokine expression from mesenteric lymph nodes in response to stimulus with various parasites. We hope to identify which difficult-to-measure aspects of immune defense can be inferred from easier measures, inferences that may generalize to other, non-model species and can be taken as a starting point for further research. And by working with re-wilded mice, we incorporate the crucial role played by antigenic experience in priming the immune system for wild animals. Our work can establish the utility of such simple ecoimmunological assays as tools for more detailed insight into immune defense by delineating the boundaries of possible inference and the suitability of such assays for particular questions.

Threespine stickleback as an emerging model for cestode-mediated immunomodulation.

Natalie Steinel (University of Massachusetts Lowell)

Models of helminthiasis are clinically valuable for both broadening our understanding of immunoregulation and aiding the development of immunosuppressive therapies. Expansion and development of new infection model systems increases our capacity to uncover the genetic factors/pathways which contribute to helminth-mediated immunosuppression. Here we present one such emerging model of model of host-parasite co-evolution and cestode-mediated immunomodulation. Threespine stickleback (Gasterosteus aculeatus) fish are commonly infected with and are the obligate intermediate host of the diphyllobothrium fish tapeworm, Schistocephalus solidus. We observed that experimental infection of stickleback leads to suppressed melanomacrophage center (MMC) responses. Work in fish and other cold-blooded vertebrates suggest MMCs are the site of humoral adaptive immunity and the proposed evolutionary precursor to the mammalian germinal center. Consistent with this hypothesis, stickleback MMCs were stained with the FDC-specific antibody, CNA42, and aggregated with splenic lymphocytes. While MMC clusters increased in size in response to immunization with T-dependent antigen, MMC size decreased in response to S. solidus infection, suggesting the cestode may have a suppressive effect on these cells. Cestodes taken from Gosling lake were capable of suppressing MMCs, while those derived from Echo Lake were not, indicating that cestode-mediated MMC suppression is cestode population dependent. Similarly, the ability of MMCs to be suppressed was fish population specific. Experimentally infected Roberts Lake fish experienced a ~30% reduction in MMC size compared to uninfected controls. On the other hand, MMCs of Gosling lake stickleback were refractory to suppression. QTL mapping of GoslingxRoberts F2 fish indicated that loci associated with variation in MMC suppression contained several genes linked to the germinal center response. Together, these results indicate that S. solidus may have a suppressive effect on the stickleback humoral response and that threespine stickleback are a viable emerging model for the study of helminth-mediated immunomodulation.

Tick-borne co-infections and immune alterations during progression of canine leishmaniosis

Breanna Scorza (University of Iowa)

Zoonotic Visceral Leishmaniasis (CanL) is driven by transmission of protozoan Leishmania infantum (Li) parasites from canine reservoirs to humans. Identifying factors driving development of severe CanL is crucial to limiting transmission and detecting novel human immune response targets.

IFNɣ-secreting CD4+ T cells are critical to control intracellular Leishmania replication and CanL progression. Although dogs can remain asymptomatic for years, we have shown immune exhaustion occurs during symptomatic CanL, with reduced CD4+ T cell proliferation and IFNɣ secretion in response to Leishmania antigen (Esch 2013). The factors controlling this immune switch remain unclear.
Our group recently identified a significant association between tick-borne pathogen co-exposure (TBC) and CanL progression (Toepp 2019). However, the immune consequences of TBC relevant to CanL progression remain to be evaluated. We hypothesize TBC causes systemic inflammation in Li-infected asymptomatic hounds, contributing to development of T cell exhaustion and symptomatic CanL. To prospectively measure the impact of TBC on CanL progression, 50 TBC seronegative dogs with subclinical Li, living in TBC areas, were randomized into blinded groups receiving oral isoxazoline tick prevention, or placebo, from 2019-2020 across two tick transmission seasons. Using peripheral blood, CD4+ T cell proliferation, inhibitory receptor expression, and cytokine production in response to Li antigen were assayed at three-month intervals. Physical examination, complete blood count, and chemistry panels were used to evaluate CanL severity according to LeishVet staging guidelines.

Combining these measurements, the association between TBC with development of immune exhaustion over time was compared in 4Dx SNAP seronegative vs. seropositive dogs. This is the first demonstration that tick-borne co-infection alters the inflammatory profile of T cells present in asymptomatic Li infection prior to progression to clinical CanL. Further, it tests the efficacy of prevention of tick infestation and subsequent disease prevents progression of CanL as a potential public health intervention.

Condensed-barcoded gRNA libraries facilitate genome-wide screening of Toxoplasma during mouse infection

Chris Giuliano (Whitehead Institute/MIT)

Genome-wide CRISPR screening has been a valuable tool for dissecting the Toxoplasma genome and uncovering genes essential for the parasite life cycle. However, most screens to date have been conducted in cell culture and therefore fail to capture many aspects of host-pathogen interaction. Some screens have included features of immune pressure, such as cytokine-stimulated macrophages, yet these environments represent only a fraction of the challenges experienced by the parasite when infecting an animal host. Mouse models of Toxoplasma infection are well-established, yet screening in mice has been limited due to the large numbers of parasites

needed to maintain library coverage, restricting screens to only a few hundred genes. To address this challenge, we have designed a condensed-barcoded gRNA-library to facilitate screening of large sets of genes in a single experiment. These libraries use few gRNAs per gene to improve library coverage, while maintaining statistical power by coupling each gRNA to an array of unique molecular identifiers (UMIs). UMIs enable tracking of individual parasite clonal lines throughout a screen and facilitate identification of bottlenecks experienced by the population. Using this approach we can reproducibly assess the dependencies of approximately 1000 Toxoplasma genes in a single mouse, making a genome-wide survey of the 8500 genes in the parasite genome readily attainable. Preliminary screens have recapitulated many known parasite genes essential for survival in the host immune environment, and uncovered additional genes with previously unknown roles in mouse infection. Characterization of these novel candidates has led to a number of insights in parasite infection biology, including features of cell-surface attachment, secreted effectors, and metabolic dependencies. In total, this work will yield the most complete survey of apicomplexan genetic dependencies during infection of an animal host to date, point to novel nodes of host-parasite interaction, and reveal possible targets for future antiparasitic therapeutic interventions.

In vitro co-infection of Leishmania infantum and Borrelia burgdorferi produces inflammatory cytokines and increased Leishmania parasite burden

Danielle Pessôa-Pereira

Canine visceral leishmaniasis (CanL) is a vector-borne zoonotic disease primarily caused by protozoan Leishmania infantum, obligate intracellular parasite classically transmitted via sand flies among reservoir host dogs and to nearby people. Our group has previously shown that exposure to tick-borne pathogens, such as Ehrlichia spp., Anaplasma spp. or Borrelia burgdorferi, can induce progression of CanL in dogs. However, how these tick-borne coinfections affect the infected myeloid cell microbicidal response against L. infantum is not known. Considering that tick-borne bacteria can interact and infect myeloid cells, we hypothesize tick-borne bacterial co-infections impact macrophage function, including cytokine production and oxidative burst, supporting L. infantum intracellular replication and survival. To investigate this, we established a co-infection model where DH82 cells, a canine macrophage cell line, were coinfected with L. infantum and B. burgdorferi – the causative agent of Lyme Disease. We found that B. burgdorferi co-infection significantly increases L. infantum parasite burden in vitro. In addition, co-infected DH82 cells displayed an enhanced inflammatory phenotype compared to controls, showing higher gene expression levels of TNFA, IL6, and IL1B. B. burgdorferi co-infection also significantly induced SOD2 gene expression – an antioxidant mitochondrial enzyme known to limit damage from reactive oxygen species (ROS) and apoptosis. These findings suggested that L. infantum and B. burgdorferi co-infection might promote robust alterations in the macrophage inflammatory response and mitochondrial ROS metabolism, to enhance L. infantum survival and replication in DH82 cells.

Session: Cellular and molecular innate immune responses

Role of IL-33 in protection from colitis due to Entamoeba histolytica

M. Jashim Uddin (Graduate Student)

Entamoeba histolytica (E. histolytica), a pathogenic protozoan parasite, causes amebic colitis, amebic liver abscess, and childhood diarrhea. Currently, the only treatment for E. histolytica infection is metronidazole, a drug which in addition to toxic side effects, is insufficient to fully eliminate parasites from the gut. Our goal is to characterize the host innate immune response to amebic infection to inform treatment. We discovered that amebic colitis was associated with induced expression of interleukin-33 (IL-33) protein and mRNA in both human and mouse colon. IL-33 is an IL-1 family cytokine implicated in protection from tissue damage and clearance of infection during bacterial, helminthic, and fungal infections. We found that treatment with recombinant IL-33 protected mice from cecal infection with E. histolytica (P=0.005 compared to PBS treated mice). IL-33 treatment also protected mice from weight loss and intestinal tissue damage. IL-33 - mediated protection was accompanied by increased expression in the colon of IL-5, IL-13, goblet cells, eosinophils, and M2 macrophages (CD206+), emphasizing the role of a type 2 immune response in IL-33 mediated protection. Administration of IL-33 protected RAG2-/- mice but not RAG2-/-ɣc-/- mice, demonstrating that IL-33 mediated protection occurred in the absence of T or B cells but required the presence of innate lymphoid cells (ILCs). Interestingly, adoptive transfer of ILC2s restored the IL-33 mediated protection in RAG2-/-ɣc-/- mice. We concluded that the IL-33-ILC2 pathway mediates protection from amebic colitis. We aim in the future to investigate the role of goblet cells and M2 macrophages downstream of IL-33.

cGAS-STING pathway activation during Trypanosoma cruzi infection leads to tissue-dependent parasite control

Natasha Perumal (Department of Cellular Biology, University of Georgia, Athens, GA, USA)

Host cell invasion by Trypanosoma cruzi is a markedly silent process, with little evidence of induction of host transcriptional changes indicative of innate immune recognition, except for a modest and reproducible type I interferon (IFN-I) response. Here we show that T. cruzi- induced IFNβ production at 24 hours post-infection was nearly abolished in primary murine cGAS-/- or STING-/- macrophages. Furthermore, infection did not limit the ability of IRF-reporter macrophages to respond to other classical agonists of the cGAS- STING pathway, suggesting that the modest IFNβ induction is a consequence of limited stimulation by T. cruzi infection rather than parasite suppression of pathway activation. Infected cGAS-/-, STING-/- and IFNAR-/- macrophages in vitro had significantly higher numbers of amastigotes compared to WT macrophages, indicating that activation of the STING pathway constrains intracellular parasite growth through the induction of interferon-stimulated genes. However, the impact of the STING pathway during infection in vivo is more complex. Despite an initial increase in parasite growth, STING-/- and IFNAR-/- mice ultimately had lower parasite burden in footpads as compared to WT mice, demonstrating a role for IFN-I expression in potentiating parasite growth at this site. However, cGAS-STING pathway activation had little impact on parasite levels in the skeletal muscle, a site of T. cruzi persistence, perhaps owing to the low basal expression of STING in myofibers. In the heart, cGAS-/- and STING-/- mice, but not IFNAR-/- mice, accumulated higher acute parasite loads, suggesting a protective role of STING sensing of T. cruzi in the heart that was independent of IFN-I. Together, these results demonstrate that host cGAS- STING senses T. cruzi infection, enhancing parasite growth at the site of entry, perhaps favoring the establishment of infection, and importantly, contributing to acute parasite control in the heart.

Mechanisms of inflammasome activation in cutaneous leishmaniasis

Christina Go (University of Pennsylvania)

Cutaneous leishmaniasis is a neglected tropical disease that can result in chronic, ulcerated lesions. Studies in leishmaniasis patients and murine models have shown that IL-1β production due to NLRP3 inflammasome activation downstream of CD8 T cell-mediated cytolysis in the lesions exacerbates disease and predicts treatment failure. Therefore, blocking signals activating the inflammasome could provide a therapeutic approach to ameliorate disease. Since these signals differ by cell type, we are characterizing the cells undergoing inflammasome activation in murine models. In one approach, we used a reporter mouse, ASC-mCitrine, a scaffolding protein that condenses into a speck upon inflammasome activation. In moderate lesions of L. major-infected mice, clusters of specks formed at the epidermis, while regions of speck formation expanded to the dermis in more severe lesions. To characterize cells by flow cytometry, we used a label specific for activated caspase-1, FAM-YVAD-FMK, and found monocytes, macrophages, and dendritic cells exhibited a low level of activated caspase-1, while a high percentage of neutrophils expressed activated caspase-1, suggesting neutrophils may the primary source of IL-1β in lesions. Finally, to determine if the target of CD8 cytolytic T cells could be an additional source of IL-1β, we utilized an in vitro co-culture model. Activated cytolytic OT1 CD8 T cells were co-cultured with ovalbumin-pulsed dendritic cells or macrophages, which led to target cell lysis and IL-1β production. Therefore, we hypothesize that cytolytic CD8 T cells drive excessive IL-1β release by inducing inflammasome activation in target cells and by the release of damage associated molecular patterns (DAMPs) during target cell lysis that activate the inflammasome in neighboring cells, such as neutrophils. Characterizing the mechanisms of IL-1β release induced by CD8 T cell target cells and signals activating the inflammasome in neutrophils will provide targets for therapeutic strategies in the treatment of immunopathologic cutaneous leishmaniasis.

Localized circuitries in cutaneous leishmaniasis that allow dermis resident macrophages to maintain M2-like properties in a strong Th1 environment

Lee, Sang Hun (LPD/NIAID/NIH)

Tissue-resident macrophages (TRMs) maintain tissue homeostasis, but they can also provide a replicative niche for intracellular pathogens such as Leishmania. How dermal TRMs proliferate and maintain their M2 properties even in the strong TH1 environment of the L. major infected dermis is not clear. Here, we show that, in infected mice lacking IL-4/13 from eosinophils, dermal TRMs shifted to a proinflammatory state, their numbers declined, and disease was attenuated. Intravital microscopy revealed a rapid infiltration of eosinophils followed by their tight interaction with dermal TRMs. IL-4–stimulated dermal TRMs, in concert with IL-10, produced a large amount of CCL24, which functioned to amplify eosinophil influx and their interaction with dermal TRMs. We also showed ILC2s control the activation and recruitment of eosinophils by secreting IL-5. Using single cell transcriptome analysis, among the alarmins that can activate ILC2, only TSLP was found to be actively transcribed in L.major infection, and was expressed mainly by dermal TRMs. Both CCL24 and TSLP expression were confined to dermis resident macrophages, implicating localized regulation of ILC2 and eosinophil by TRM to maintain its M2-like phenotype in inflammatory settings.

IgM is required for expansion of macrophages in the pleural cavity during Litomosoides sigmodontis infection

Lucy Jackson-Jones (Lancaster University)

The influence of IL-4 on macrophage metabolism has been the focus of many studies of bone-marrow derived and peritoneal macrophages. In contrast, the influence of in vivo exposure to IL-4 on the metabolic profile of pleural cavity macrophages has not been determined. IgM is the first antibody produced during an immune response, the secreted form is pentameric and often polyclonal. Within the pleural cavity, natural antibodies are secreted by innate-like B-cells residing within FALCs of the pericardium & mediastinum. Natural IgM recognizing oxLDL has been associated with increased protection from morbidity and mortality in atherosclerosis. Whether locally produced IgM auto-antibodies that recognise lipoproteins have a role in the function of macrophages in other contexts is under investigated. Here we investigated the function of IgM in the regulation of resident pleural macrophage function during type-2 inflammation. During infection with the tissue tropic filarial worm Litomosoides sigmodontis (Ls), IgM increases locally within the pleural fluid of experimental mice. Using Ls infection of sIgM deficient mice, we find that the number of pleural macrophages fails to increase when compared to IgM sufficient mice; reduced pleural macrophage number in sIgM deficient mice correlates with reduced killing of L3/L4 stage larvae. Analysis of the effect of in vivo delivery of exogenous IL-4c on pleural macrophages showed that macrophages from sIgM deficient mice are lipid laden, do not accurately complete cell cycle, fail to increase cholesterol biosynthesis and oxygen consumption. Delivery of IL-4c alone is able to increase local IgM antibody secretion into the pleural fluid of wild type mice including increases in local IgM anti-oxLDL autoantibodies. While wild type pleural macrophages are able to internalize excess lipoprotein particles, pleural macrophages from sIgM deficient mice are not. Collectively these results suggest that natural IgM protect wild-type pleural macrophages from lipid overload.

Trichinella spiralis-induced mastocytosis and erythropoiesis are simultaneously supported by a bipotent mast cell/erythrocyte precursor cell

Christina M. Hernandez (Rutgers University, NJMS)

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 erythrocytes and mast cells. Our previous studies have recently identified a population of hematopoietic progenitor cells (HPCs) that possess enhanced mast cell potential, traffic to inflamed tissues, and contribute to host protection following a helminth challenge. In addition, we have previously reported that helminth-activated HPCs exhibit increased expression of the metabolic enzyme carbonic anhydrase 1 (Car1). Although the exact functions of Car1 remain to be fully defined, it has been reported to play an important role in erythrocyte development. Here, we employed unbiased single-cell RNA sequencing and identified that Car1 marks a distinct bone marrow-resident progenitor population in both mice and humans. Further, we generated a novel Car1-reporter mouse model and revealed that Car1-expressing progenitor cells represent a bipotent erythrocyte and mast cell precursor population. Finally, we show that Car1-expressing cell populations are mobilized following a Trichinella spiralis infection. Collectively, these data suggest that Car1-expressing HPCs represent a committed mast cell/erythrocyte precursor population that traffics to inflamed tissues and possess the potential to promote both worm expulsion and wound healing events following a helminth infection.

Session: T cell responses

Effector function prior to establishment of the phagosomal pathogen niche is required for protective CD4+ T cell-mediated immunity against Leishmania

Leah S. Hohman (University of Calgary)

Leishmania represents an appealing model organism to study CD4+T cell-mediated protective immunity against phagosomal pathogens and features localized primary and secondary infection sites with defined innate and adaptive responses. Upon secondary challenge of chronic L. major-infected C57Bl/6 mice, rapid delivery of CD4+T effector (TEFF) function via IFN-g-mediated activation of infected monocytes is associated with optimal immunity. However, the absolute requirement for immediate effector function has yet to be demonstrated. Thus, we isolated time as a variable in the delivery of Ly6C+CD4+TEFF-mediated effector function. We adoptively transferred (AT) chronic mouse-derived Ly6C+CD4+TEFFs into naïve recipients immediately (D0) or 4 days (D4) post-L. major challenge. In this time window Leishmania establishes an intracellular niche but does not proliferate. At day 21 post-challenge, D4 AT resulted in a total loss of the parasite control mediated by D0 transfer. Dose titration of Leishmania-specific Th1 TcR-Tg T cells revealed that no number of TcR-Tg Th1 cells transferred at D4 overcame the requirement for rapid D0 immunity. To address whether parasite niche establishment

modulated Th1 TEFF cell recruitment, intravital imaging was employed. Ag-sp T cells were present in the infected dermis at significantly lower numbers following D4 vs D0 transfer at 4 days post-T cell transfer, indicating a recruitment deficit. Rapid CD4+ Th1 effector function was required for circulating Th1 TEFF cells to capitalize on an early recruitment window associated with cxcl9 and cxcl10 expression, to control parasite burden, and to prevent parasite niche establishment characterized by altered cell recruitment, gene expression, and functional capacity of both innate and adaptive immune cells. We propose that near-immediate effector function mediated by circulating TEFF cells is required to prevent immunomodulation of permissive monocytes by Leishmania and represents an important consideration for prophylactic vaccination against phagosomal pathogens reliant on Th1 immunity.

Transgenic T cell epitope-expressing Strongyloides ratti reveals that helminth-specific CD4+ T cells constitute both Th2 and Treg populations

Bonnie Douglas (University of Pennsylvania)

Helminths are distinct from microbial pathogens in both size and complexity, and are the likely evolutionary driving force for type 2 immunity. CD4+ helper T cells can both coordinate worm clearance and prevent immunopathology, but issues of T cell antigen specificity in the context of helminth-induced Th2 and T regulatory cell (Treg) responses have not been addressed. Herein, we generated a novel transgenic line of the gastrointestinal nematode Strongyloides ratti expressing the immunodominant CD4+ T cell epitope 2W1S as a fusion protein with green fluorescent protein (GFP) and FLAG peptide in order to track and study helminth-specific CD4+ T cells. C57BL/6 mice infected with this stable transgenic line (termed Hulk) underwent a dose-dependent expansion of activated CD44hiCD11ahi 2W1S-specific CD4+ T cells, preferentially in the lung parenchyma. Transcriptional profiling of 2W1S-specific CD4+ T cells isolated from mice infected with either Hulk or the enteric bacterial pathogen Salmonella expressing 2W1S revealed that pathogen context exerted a dominant influence over CD4+ T cell phenotype. Interestingly, Hulk-elicited 2W1S-specific CD4+ T cells exhibited both Th2 and Treg phenotypes and expressed high levels of the EGFR ligand amphiregulin, which differed greatly from the phenotype of 2W1S-specific CD4+ T cells elicited by 2W1S-expressing Salmonella. Altogether, this new model system suggests effector as well as immunosuppressive and wound reparative roles of helminth-specific CD4+ T cells. This work establishes a new resource for studying the nature and function of helminth-specific T cells.

PD-L1 – PD-1 interactions limit effector Treg cell populations at homeostasis and during infection

Joseph Perry (University of Pennsylvania)

While much is known about the factors that promote the development of diverse Treg cell responses, less is known about the pathways that constrain Treg cell activities. The studies presented here reveal that at homeostasis there is a population of effector Treg cells that express PD-1, and that blockade of PD-L1 or loss of PD-1 results in increased Treg cell activity. In response to infection with the parasite T. gondii, the early production of IFN-ɣ results in widespread upregulation of PD-L1. Moreover, blockade of PD-L1, whole body deletion of PD-1, or lineage specific deletion of PD-1 in Foxp3+ cells prevented the loss of the effector Treg cells but resulted in reduced pathogen specific CD4+ T cell responses during infection. Thus, at homeostasis basal PD-L1 expression constrains and tunes the pool of Treg cells, but during infection the upregulation of PD-L1 provides a mechanism to contract the Treg cell population required to maximize the development of pathogen specific CD4+ T cell responses.

The impact of antigen dose on the generation, function and longevity of Leishmania-specific CD4+ memory T cells

Zhirong Mou (University of Manitoba)

There is currently no approved vaccine against human cutaneous leishmaniasis (CL). This is due in part to insufficient knowledge regarding the immunodominant Leishmania antigens, the nature of T cells that respond to them and the factors that regulate the generation, function and longevity of different subsets of memory T cells against these antigens. Antigen dose is known to influence the magnitude and quality of the host immune response. Specifically, antigen dose has been shown to regulate the differentiation of naïve CD4+ T cells into Th1 effector cells that mediate resistance to CL. However, the influence of antigen dose on the magnitude and quality (cytokine production and function) of Leishmania-specific CD4+ T cells memory response is unknown. Recently, we identified Leishmania phosphoenolpyruvate carboxykinase (PEPCK) as protective antigen and showed that PEPCK335–351 peptide is the dominant CD4+ T cell epitope. We generated PEPCK-specific TCR transgenic (PEG) mice and found that >90% of their CD4+ T cells express PEPCK-specific TCR. We found that PEG CD4+ T cells specifically respond to PEPCK in vitro and in vivo. Upon adoptive transfer into congenic mice followed by

immunization with different doses (0.05, 0.5 or 5 nmol) of PEPCK335-351 peptide, PEG cells proliferated robustly and produced IFN-ɣ in a dose-dependent manner. Interestingly, high dose (5 nmol) PEPCK peptide induced higher frequency of poly-functional (IFN-ɣ+IL-2+TNF+) cytokine producing PEG cells and this was associated with higher expression of CD44, CCR7 and CD62L, markers that characterize central memory (Tcm) phenotype. Moreover, PEG cells in mice immunized with high dose PEPCK peptide showed higher IFN-ɣ recall response following secondary in vivo peptide or L. major challenge. This higher IFN-ɣ response was associated with better protection against virulent L. major challenge. Collectively, these findings show that antigen dose influences the generation, magnitude and function of Leishmania-specific CD4+ memory T cells.

Helminth-driven fetomaternal crosstalk primes regulatory networks to modify inflammatory T cell responses.

Matthew Lacorcia (Technical University of Munich, Institute for Medical Microbiology, Immunology, and Hygiene, Munich, Germany)

Question Prenatal exposure to infections can modify immune development. Environmental disturbances during early life alter the incidence of inflammatory disorders and priming of immune responses. Infection with the helminth Schistosoma mansoni is studied for its ability to alter immune responsiveness, and associated with variations in co-infection, allergy, and vaccine efficacy in endemic populations. Exposure to maternal schistosomiasis during early life, even without transmission of infection, can result in transgenerational effects on immune responses to bystander antigenic challenges, as we have previously shown with allergic asthma in a mouse model. This study explores the immunological priming effects of maternal helminth infection during pregnancy, as relate to allergic responsiveness and vaccination mediating by T cell activation. Methods: We employ a long-term chronic murine model of maternal schistosomiasis to evaluate effects on modified inflammatory T cell biology. Steady state analysis of immune priming within these offspring was coupled to functional assays including in vivo allergic and immunization models, anti-viral vaccination and challenge, as well as in vitro assays. Results: Maternal schistosomiasis altered CD4+ responses during allergic sensitization and inflammation in lungs, with skewed IL-4/B-cell-dominant response to antigenic challenge in priming lymph nodes. CD8+ T cell responses was also altered during immunization, dependent upon vaccine formulation, and modified efficacy of vaccination against viral infection in a murine Hepatitis B virus model. Modified CD8+ responses were associated with an altered dendritic cell phenotype sustained into adulthood, providing evidence for complex priming effects imparted by infection via fetomaternal crosstalk. Conclusions: We observed that transgenerational imprinting can modify T cell responses, altering sensitivity to unrelated allergens as well as limiting protective antiviral vaccine efficacy. Mechanistically, we identify a regulatory network consisting of a deviated IL-4/B-cell axis and a modified DC phenotype sustained into adulthood, with evidence of innate training pointing to complex immunological interactions imprinted during early life exposure.

MicroRNA-21 deficiency promotes the early Th1 immune responses and resistance towards visceral leishmaniasis

Erin Holcomb, Sanjay Varikuti (The Ohio State University)

MicroRNA-21 (miR-21) is known to inhibit IL-12 expression and impair the development of a Th1 immune response necessary for control of Leishmania infection. It has been recently shown that Leishmania infection induces miR-21 expression in dendritic cells and macrophages and inhibition of miR-21 restores IL-12 expression. Since miR-21 is known to be expressed due to inflammatory stimuli in a wide range of hematopoietic cells, we investigated the role of miR-21 in shaping innate and adaptive immune responses during Leishmania donovani infection. We found that miR-21 expression was significantly elevated in dendritic cells, macrophages, inflammatory monocytes, PMNs, and in the spleen and liver tissues following Leishmania donovani infection, concomitant with an increased expression of IL-6 and STAT3. BMDCs from miR-21 null mutant mice showed an increased production of IL-12 and decreased production of IL-10. Further, upon L. donovani infection, miR-21 KO mice exhibited significantly higher numbers of IFN-ɣ and TNF-α producing CD4+ and CD8+ T cells in both livers and spleens, that was accompanied by increased production of Th1-associated IFN-ɣ, TNF-α, and Nitric Oxide (NO) from the splenocytes. Finally, miR-21KO mice showed significantly higher numbers of developing and mature hepatic granulomas resulting in reduced parasitic loads in the livers and spleens compared to similarly infected wild type mice. These observations suggest that induction of miR-21 results in exacerbation of this disease and also confirms the direct suppressive role of miR-21 on IL-12 and IL-12 induced IFN-ɣ associated Th1 immune reactions. In conclusion, miR-21 promotes susceptibility to L. donovani infection by complimenting IL-6, STAT-3 signaling, and inhibiting the development of Th1 immune responses and can therefore be used as a potential target for the resolution of VL disease.

Heterogeneity of Tregs in Helminth Infection

Caitlin McManus (University of Glasgow)

Approximately 1.5 billion people worldwide are infected with helminths. However, many individuals are asymptomatic and appear to tolerate the infection, correlated with an increase in regulatory cells such as Tregs. While this expansion is often associated with the tolerant state, the role that Tregs play between resistance and susceptibility to helminth infection is not fully understood. Moreover, despite increasing recognition of the heterogeneity of Tregs in vivo, little is known of the composition of the Treg response to helminths, or of their interactions with T effector cells. To address this, we use Heligmosomoides polygyrus, a rodent parasite which infects the small intestine, to study the dynamics of the T cell response to helminth infection. In this model, host genetics influence susceptibility to infection, whereby C57BL/6 mice are susceptible to infection whereas BALB/c mice are partially resistant. We compared the T cells of H. polygyrus infected and naïve BALB/c and C57BL/6 mice via flow cytometry and single cell sequencing which allowed us to identify 6 potential subtypes of Tregs, defined as (1) Bcl2+, (2) CD28+, (3) Ly6E+, (4) cMaf+, (5) IL7R+ and (6) ICOS+CTLA-4+. In both strains there is Treg expansion, however they differ in the degree of expansion and the proportions of different Treg subtypes. In particular, Ctla4 and Icos, which balance CD28 mediated co-stimulation and increase the suppressive capability of Tregs are expressed at a higher level in susceptible C57BL/6 mice, and are selectively upregulated on Tregs and not the effector CD4+ population. From these data we can hypothesise which specific Treg subsets are most responsible for promoting susceptibility to H. polygyrus infection in the mouse model, and begin to speculate about their role in regulating immune responses against human helminthiases.

Session: Human Immunology

Effect of hookworm infection and anthelmintic treatment on naturally acquired antibody responses against the GMZ2 malaria vaccine candidate and constituent antigens

Benjamin Amoani (UNiversity of Cape Coast)

Background: Malaria and helminths diseases are co-endemic in most parts of sub-Saharan Africa. Immune responses from each of these pathogens interact, and these interactions may have implications on vaccines. The GMZ2 malaria vaccine candidate has recently showed modest efficacy in a phase IIb multicenter trial. Here, we assessed the effect of hookworm (Necator americanus) infection and anthelmintic treatment on naturally acquired antibody responses against GMZ2 and constituent antigens.

Methods: This cross-sectional study was conducted in the Kintampo North Municipality of Ghana. Blood and stool samples were taken from 158 individuals (4–88 years old) infected with either P. falciparum alone (n=59) or both hookworm and P. falciparum (n=63) and uninfected endemic controls (n=36). Stool hookworm infection was detected by the Kato-Katz method and PCR speciation. Malaria parasitaemia was detected by RDT, light microscopy and P. falciparum-specific 18S rRNA gene PCR. Serum samples were obtained prior to hookworm treatment with a single dose of albendazole (400 mg) and three weeks (21 days) after treatment. Levels of IgG1, IgG3 and IgM against GMZ2, MSP3 and GLURP R0 were measured by ELISA and compared among the groups, before and after treatment.

Results: Participants with P. falciparum and hookworm co-infection had significantly higher IgG3 levels to GMZ2 than those with only P. falciparum infection and negative control ($p<0.05$) at baseline. Treatment with albendazole led to a significant reduction in IgG3 levels against both GMZ2 and GLURP R0. Similarly, IgM and IgG1 levels against MSP3 also decreased following deworming treatment.

Conclusion: Individuals with co-infection had higher antibody responses to GMZ2 antigen. Treatment of hookworm infection resulted in a significant reduction in antibody responses against GMZ2 and constituent antigens. Thus, hookworm infection and treatment could have a potential implication on malaria vaccine efficacy.

Early reduction in PD-L1 expression predicts faster treatment response in human cutaneous leishmaniasis.

Nidhi S. Dey (York Biomedical Research Institute, Hull York Medical School, University of York)

Cutaneous leishmaniasis (CL) is a chronic disfiguring disease caused by Leishmania parasites. Pentavalent antimonials (e.g. sodium stibogluconate; SSG) are first line drugs for CL, despite protracted and painful treatment regimens. The disease is characterized by an inability to clear skin parasite load and chronic inflammation. Effectiveness of T cell responses to Leishmania that are essential for parasite clearance and healing may be hindered by the presence of immunoregulatory molecules and / or by T cell exhaustion. Additionally, data from animal models indicate that the efficacy of SSG requires drug-immune synergy, but mechanistic insight from patients is lacking. Here, we studied FFPE skin biopsies from patients in Sri Lanka with CL due to L. donovani infection, both at presentation and early after

the initiation of drug treatment. Immune-targeted transcriptomics revealed reduced expression of immune checkpoint expression on treatment. We confirmed reduced expression of both PD-L1 and IDO1 on a second validation cohort using digital spatial profiling and quantitative immunohistochemistry. Using dual IHC-FISH, we show that intra cellular parasitism increases the expression of both IDO1 and PD-L1 on CD68+ monocytes/macrophages. Crucially, early reduction of PD-L1 but not IDO-1 was predictive of faster cure rate and occurred in parallel with reduction of parasite load. Multivariate cox proportional hazard model showed that patients with lower PD-L1 expression during treatment were 5 times more likely to cure earlier. Our data support a model whereby the initial anti-leishmanial activity of antimonial drugs alleviates checkpoint inhibition of T cell immunity, facilitating immune-drug synergism and clinical cure. Our findings demonstrate that PD-L1 expression can be used as an early predictor of faster clinical response to SSG treatment and support the use of PD-L1 inhibition as adjunct host directed therapy in CL.

Immune Responses in Toxoplasmosis and Malaria Co-Infections among Residents of Some Rural Communities in Southwestern, Nigeria

Maureen Efenovwe (University of Ibadan, Department of Zoology (Parasitology unit))

Background: Malaria and toxoplasmosis have similar cellular and biochemical pathways known to modulate immune responses, which alter the pathophysiology and immunological presentations in the diseases. Co-infections of Plasmodium and Toxoplasma gondii lead to competitive establishment by the parasites. The study was aimed at determining the immune responses to co-infections of malaria and toxoplasmosis among residents in Akinyele, rural southwest Nigeria

Method: In a cross-sectional survey, blood samples were collected from 192 volunteers, and analysed by microscopy for Plasmodium falciparum. Antibodies (IgG and IgM) to Toxoplasma gondii MIC 3 protein and cytokines (IL2, IL6, IL10 and IL12) were also analysed. PCV, and other anthropogenic factors were measured as indices parasite associated morbidities.

Result: The prevalence of co-infection of the parasites was 20.4%. Toxoplasma seroprevalence was 27.5% for IgG, 8.98% for IgM, and 2.6% for both IgG and IgM. Malaria prevalence was 72.9%. Plasmodium intensity was highest in people below 20 years while Toxoplasmosis was more prevalent in those 51-60 years. Toxoplasma seropositivity, malaria prevalence and Plasmodium intensity were significantly higher ($P<0.05$) in females. Presence of IgG and IgM antibodies to Toxoplasma was associated with increased ($P<0.05$) and decreased Plasmodium intensity respectively. Peripheral IL-2 levels were higher (59.02±0.19pg/ml) in co-infection of malaria and Toxoplasma IgG seropositive individuals while IL-10 was significantly ($P<0.05$) elevated in malaria co-infection with IgM seropositive individuals. IL-6 levels increased ($P<0.05$) with severity of malaria while IL-2 was lowest in severe malaria. Anameia was observed in 12.4% of the participants, 13.6% of these were seropositive. Elevated IL-6 and decreased IL-12 levels were observed with anaemia.

Conclusion: Active Toxoplasma gondii co-infection with malaria may suppress malaria pathology while Plasmodium and chronic T. gondii co-infection may lead to increased production of the pro-inflammatory cytokine IL-2. Malaria co-infection did not have any effect on anaemia severity.

Adoptive transfer of helminth antigen stimulated human PBMCs attenuates disease progression in a humanised mouse model of graft-versus-host disease

Alison Aldridge & Sandra O'Neill (Dublin City University)

There is strong evidence demonstrating that molecules derived from parasitic worms significantly downregulates major pro-inflammatory pathways, reducing inflammation and opening up the possibility of these molecules as treatments for a whole spectrum of inflammatory disorder. Fasciola hepatica is a trematode worm that causes fascioliasis, a neglected tropical disease in humans and livestock. Studies have demonstrated the beneficial effects of F. hepatica molecules in murine models of allergy, arthritis, colitis, multiple sclerosis, sepsis and type I diabetes. Here we investigated the immunomodulatory properties of the parasite's tegumental coat (FhTeg), a major antigen source in a humanised mouse model of host versus graft disease (HvGD). Previous studies demonstrated that FhTeg induces a novel phenotype of dendritic cells that induce anergic CD4+ T-cells. In this study, FhTeg binds to and modulates cytokine production in human PBMCs, in particular targeting the CD4+ population resulting in reduced levels of TNF, IL-2 and IFNγ and increased markers of anergy. Furthermore, the adoptive transfer of FhTeg stimulated PBMCs to HvGD mice attenuated disease progression by increasing survival and reducing pathological scores. These mice also displayed a significant decrease in the total number of human CD4+ cells expressing TNF, IL-2 and IFNγ in the spleen, liver and lung. This study therefore concurs with other studies demonstrating the immune modulatory effects of helminth antigens. It also suggests that anergic CD4+ T cells are associated with successful Fasciola hepatica infection and highlights an important role for FhTeg in contributing to the overall immunosuppressive effects of this parasite.

CD8+CD57+T cells in the pathogenesis of disseminated leishmaniasis

Thiago Cardoso, Cayo Abreu (Researcher in Public Health of IGM-Fiocruz)

Disseminated leishmaniasis (DL) caused by L. (Viannia) braziliensis (L.b), is an emerging clinical form of tegumentary leishmaniasis, characterized by the presence of 10-1000 papular, acneiform and ulcerated lesions, in two more sites on the body. CD8+T cells are very important role in the inflammatory response and progression of DL. Peripheral blood mononuclear cells (PBMC) and biopsies from patients with cutaneous leishmaniasis (CL) and DL were used to performed: CD8 T cells sorted, differentiation of monocytes into macrophages (MØ) to co-cultures and PBMC cultures stimulated with SLA (soluble L.b antigen). Co-cultures with autologous CD8+T cells (5: 1 CD8: MØ) were performed with uninfected and infected MØ. Biopsies also were cultured for 72 hs to determine the profile of CD8+T cells by flow cytometry and supernatant´s cytokines measurement. Statistical analysis were performed by Mann-Whitney and Kruskal-Wallis test, post-tested by Dunn's. Here we shown a higher frequency of CD8+CD107a+T cells in peripheral blood and biopsies from DL and these cells shown CD57+ expression, determining a degranulation and exhaustion profile. After co-cultures, CD8+T cells from DL shown high granzyme B (GzB) expression. Apparently, specific CD8+CD57+T cells, through cytotoxic action, can promote lysis of infected cells that consequently lead to the release of intracellular amastigotes, contributing to the spread of the parasite in DL.

Interleukin 17 stimulates mononuclear cells to kill Echinococcus granulosus by NO-dependent mechanism: immuomodulation by laminated layer

Manel Amri, Sara Benazzouz, Fahima Ameur, Insaf-Meriem Boutemine, Samia Bouaziz, Imene Soufli, Houda Belguendouz and Chafia Touil-Boukoffa ("Cytokines and NOSynthases", Laboratory of Cellular and Molecular Biology (BCM), Faculty of Biological Sciences (FSB), University of Sciences and Technology Houari Boumediene (USTHB))

Background: Human echinococcosis is one of the world's major zoonotic infections. Dissemination of protoscoleces (PSC, cystic components) during surgery constitutes a source of relapse. We have highlighted an evident role of laminated layer (LL, outer layer of cyst) in PSC protection against IFN-ɣ/NOS2 (Nitric Oxide Synthase 2) pathway by induction of Arginase. Discovery of the Th17 cell lineage and functions in immune responses of man prompted us to investigate the role of IL-17 in host defense during human echinococcosis. Method: We have investigated the effect of IL-17, corresponding antibody and LL extract on PBMC (mononuclear cells) cultures and PSC co-cultured with PBMC of hydatic patients (before and after surgery) and healthy donors. After 20h, NO, Urea and Arginase activity were evaluated. PSC viability was also evaluated in all cocultures.

Results: Our results demonstrated that IL-17 decrease protoscoleces viability ($p<0.0001$). Moreover, we observed a concomitant elevation of NO levels ($p<0.0001$) and a decrease in Urea levels ($p<0.001$) and Arginase activity. Interestingly, the use of anti-IL-17 and LL extract enhanced protoscoleces survival. This effect is associated with a decrease in NO level and an elevation in Urea level and Arginase activity. Similar findings are observed in the cases of PBMC of patients and healthy donors.

Conclusion: The results reported here show that IL-17 plays a relevant role in the protective immune response during human echinococcosis. This role may be mediated by NOS2 up-regulation and Arginase down-regulation. LL seems to have a protective effect on the parasite. Our findings provide useful tools for development of therapeutic strategies.

Session: Mucosal and barrier tissue immunology

Helminths battle type 2 immunity for control of the intestinal stem cell niche

Danielle Karo-Atar (McGill University)

Intestinal helminths remain one of the most pervasive parasites of the animal kingdom by stimulating host defense pathways that prioritize tissue adaptation over parasite expulsion. Although helminths form intimate interactions with the intestinal epithelium, their ability to directly shape the fate of this barrier tissue is unknown. Here we show that infection of mice with Heligmosomoides polygyrus bakeri (Hpb) induces a fetal reprogramming of the intestinal stem cell niche coincident with adult parasite adherence to intestinal villi. This reprogramming event is characterized by a regenerative Hippo pathway transcriptional signature and the emergence of Clusterin-expressing 'revival' stem cells (revSC) previously shown to drive intestinal repair following acute injury. Furthermore, lineage-tracing studies confirm the presence of revSC-derived progeny along the villi of Hpb-colonized animals. Remarkably, intestinal organoids exposed to Hpb excretory-secretory products rapidly assume a spheroid morphology and express a fetal gene battery, a phenotype associated with gut organogenesis. By contrast, interleukin-13 inhibit revSC development and the fetal gene program in vitro while deletion of

type 2 cytokine signaling in vivo lead to an enhanced fetal host response, increased host susceptibility to infection and improved worm fitness. Collectively, our study reveals how a helminth parasite co-opts a tissue development program to counter type 2 immune-mediated expulsion and maintain chronic infection.

Deletion of Matrix Metalloproteinase 17 renders Mice resistant to chronic Trichuris muris Infection by affecting epithelial Goblet Cell-specific Immune-effectors

Pia Vornewald (NTNU – Norwegian University of Science and Technology)

The intestinal smooth muscle (ISM) is important for moving food along the gut by contractile peristaltic waves. Increased gut peristalsis is also a classic part of type 2 immune responses against parasitic nematode infections to aid in expulsion. Despite the importance of the peristaltic role of ISM in intestinal biology, little is known about other potential roles of the ISM. We recently found that smooth muscle cells can act as modulators of intestinal epithelial regeneration and the intestinal stem cell niche by secreting specific factors*. In part, this was dependent on the expression of the smooth-muscle specific matrix metalloproteinase 17 (MMP17). In the current study, we focus on how MMP17 may be involved in immunity to (helminth) infection.

Using a KO mouse model, we challenged Mmp17-/- mice with both bacterial and helminth infections (Citrobacter rodentium and Trichuris muris). We found no differences in the ability to mount appropriate immune responses, as loss of MMP17 did not alter immunity to C. rodentium or high-dose T. muris infection. However, unlike WT control mice, Mmp17-/- mice were resistant to chronic T. muris infection. Taking a closer look at the epithelium using RNA-seq and immune histochemistry, we discovered differences in goblet cell effector responses, such as increased levels of RELM-beta, under naïve conditions. We used organoids to identify that raised levels of goblet cell effector proteins are not epithelial intrinsic in Mmp17-/- tissue, but rather a consequence of the surrounding niche.

With this work we show that ISM is involved in immunity to infection not only by its peristalsis function. In our ongoing research, we are trying to discover through what mechanisms ISM-derived MMP17 influences the intestinal epithelial effector processes in immunity to infection.

Cysteinyl leukotriene receptor-1 is required for clearance of Nippostrongylus brasiliensis and development of protective memory responses during secondary infection.

Paballo Mosala (University of Cape Town)

Cysteinyl leukotrienes (cysLTs) are inflammatory lipid mediators that play a major role in pathophysiology of inflammatory diseases. CysLTs signal primarily through cysteinyl leukotriene receptor-1 (cysLTR1) and have been reported for their ability to drive Th2 immune responses. Initiation and amplification of robust Th2 immune responses such as inflammation, mucous secretion and smooth muscle contractions is crucial for conferring protective immunity to Nippostrongylus brasiliensis infection in mice. We therefore hypothesized that cysLTs signaling through cysLTR1 could play a crucial role in termination and resolution of N. brasiliensis adult worms during primary and secondary infection. CysLTR1-/- mice infected with 500 L3 N. brasiliensis larvae, treated, re-infected and killed at day 5 post re-infection were biopsied for analysis of pathology and immune responses. Our results indicated increased worm burden at day 7 and a delay in clearance of adult worms by day 9 post infection in cysLTR1-/- mice during primary infection. The delay was associated with reduced Th2 immune response as judged by reduced production of IL-4, IL-5, and IL-13 in the intestine and impaired smooth muscle contractions of the intestine. Interestingly, there was sufficient mucous producing cells despite the reduced IL-13 production. Re-infection with N. brasiliensis resulted in impaired worm expulsion in cysLTR1-/- mice as compared to littermate control mice, additionally, there was a reduction in IL-13 production in lungs and draining lymph nodes in infected cysLTR1-/- mice as compared to littermate control mice. Furthermore, reduced recruitment of effector CD4+ T cells and central memory CD4+ T cells in the lungs of cysLTR1 deficient mice compared to control mice was noted. Taken together, our presentation will unprecedentedly describe how cysteinyl leukotrienes signaling via cysLTR1 is essential for conferring host protection during secondary N. brasiliensis infection.

IL-11 regulates innate mucosal immunity in acute helminth infection

Jonah Kupritz (Laboratory of Parasitic Diseases, NIAID, National Institutes of Health)

Interleukin (IL)-11, a pleiotropic IL-6 family-member cytokine, appears 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 the lungs of Ascaris-infected mice at 8 days post-infection (dpi), we sought to understand the role played by IL-11 at lung

barrier surfaces following infection with Ascaris. Compared to uninfected mice, IL-11 levels were significantly elevated in the lung tissue of Ascaris-infected mice (2,196 pg/mL vs 968 pg/mL, p<0.001 at 8 dpi). Using both flow cytometry and confocal microscopy, we found that bronchial epithelial cells and subepithelial fibroblasts were the primary IL-11-producing populations following Ascaris infection. To assess the function of IL-11 in Ascaris infection, we administered anti-IL-11 antibody intranasally during Ascaris infection. This IL-11 neutralization impaired the influx of macrophages and neutrophils to the lungs; moreover, there was induction of CXCL5, a chemokine known to inhibit neutrophil trafficking. Intranasal administration of recombinant IL-11, conversely, induced G-CSF and CXCL1, increased neutrophil influx, and reduced airway inflammation and worm burden (40% decrease, P<0.01) in the Ascaris-infected mice when compared with PBS-treated, infected mice, suggesting an immune-mediated regulatory effect of IL-11 in helminth infection; similar effects have been reported in bacterial pneumonia and inflammatory bowel disorders. To model the interaction between Ascaris and lung epithelial cells, we used an in vitro system whereby a human bronchial epithelial cell line grown in a monolayer on an extracellular gel matrix was shown to produce markedly increased (55% above baseline) amounts of IL-11 following exposure to Ascaris larvae. These data suggest that IL-11 acts as an alarmin released by bronchial epithelial cells in response to Ascaris larvae (or larval products) that may in turn regulate the neutrophil-dominated inflammation seen during acute helminth infections.

Basophils promote tissue-resident optimal Th2 cell function during helminth infection

Lauren Webb (University of Washington)

Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and promotes clearance of gastrointestinal helminths, which infect over 2 billion people worldwide. Basophils are a rare innate immune cell population that have previously been suggested to interact with CD4+ T cells during helminth infection, however, how these interactions might influence CD4+ T cell function is not well understood. During infection with the helminth Trichuris muris, CD4+ T cells are an essential source of type 2 cytokines that promotes parasite clearance. While the molecular mechanisms involved in driving basophil function were previously unclear, we have recently shown that during T. muris infection, the Notch signaling pathway regulates basophil gene expression programs during infection and drives basophil localization proximal to CD4+ T cells in the inflamed intestine. Consequently, mice lacking basophil-intrinsic Notch signaling had reduced Gata3+ T helper 2 cells at the site of infection and decreased type 2 inflammation following T. muris infection, resulting in impaired worm clearance. We are now investigating the role of productive basophil-T cell interactions in driving fulminant type 2 immunity in the intestine. These studies highlight that blockade of Notch signaling in basophils inhibits infection-induced gene expression changes in CD4+ T cells and limits their viability in vivo, while in vitro coculture experiments demonstrate that basophils can directly drive a Th2 phenotype and function in CD4+ T cells from T. muris-infected mice. Thus, a deeper understanding of the role of basophils in driving Th2 cell function has the potential to facilitate the development of targeted therapies against both innate and adaptive type 2 immune mechanisms.

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. Understanding of the persistence mechanisms is lacking, but 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 in vivo. In the presented study, 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 monocyte-derived 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 polyclonal Treg controls. Co-culture of PEPCK-specific CD4+ T cells, L. major-infected monocyte-derived 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. We are currently characterizing whether effector T cell responses are altered in healed lesions, where persistently-infected cells are readily observed. 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.

POSTERS

Poster Session 1

P1

Super-resolved view of PfCERLI1, a rhoptry associated protein essential for Plasmodium falciparum merozoite invasion of erythrocytes

Sonja Frolich (Research Centre for Infectious Diseases, The University of Adelaide)

The disease-causing blood-stage of the Plasmodium falciparum lifecycle begins with invasion of human erythrocytes by merozoites. It involves the coordinated release of ligands from specialised invasion organelles, the micronemes and rhoptries, which secrete parasite proteins onto or within host red blood cell (RBC) to prime attachment, mechanical entry and establishment of an intracellular environment suitable for parasite growth. Limited evidence to date suggests that prior to RBC entry, the narrow top of the two rhoptries are required to fuse to the merozoite plasma membrane before neck contents can be released and the irreversible point of attachment to the RBC forms (the tight-junction). As invasion proceeds, fusion of the two rhoptries commences at the neck and continues to the bulb before the structure partially collapses to facilitate release of rhoptry contents. Despite the importance of the protein network orchestrating the rapid (~10 seconds), multi-step, process of rhoptry fusion and content release, the proteins involved are largely uncharacterised. Recently, we utilised knockdown studies and identifed an essential role for the conserved protein P. falciparum Cytosolically Exposed Rhoptry Leaflet Interacting protein 1 (PfCERLI1) in rhoptry function. Using biochemical techniques and quantitative super-resolution microscopy, we show that PfCERLI1 localises to the cytosolic face of the rhoptry bulb membrane and knockdown of PfCERLI1 inhibits merozoite invasion. While schizogony and merozoite organelle biogenesis appear normal, reduction in PfCERLI1 expression appears to block secretion of key rhoptry antigens that coordinate merozoite invasion. While further studies need to be undertaken to determine the fine detail of how PfCERLI1 knockdown causes these changes in rhoptry function, identification of PfCERLI1's direct association with release of rhoptry antigens is a key step in understanding the complex molecular events that control rhoptry secretion during invasion. This study makes extensive use of semi-automated quantitative immunofluorescence microscopy and highlights how this powerful tool can be used to study the process of invasion.

P5

CD18 regulates monocyte progenitors proliferation and differentiation into alternatively activated macrophages during chronic schistosomiasis

Camila Souza (Department of Clinical Analyses, Toxicology and Food Science, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo)

Infection with Schistosoma mansoni causes a chronic parasitic disease that progresses to severe fibrosis, and eventually death. Along the course of schistosomiasis, innate immune cells are recruited and accumulate into Th2-granuloma in the liver. Recently, our group demonstrated that the common chain of β2-integrin (CD18) was crucial for monocytopoiesis and resistance to experimental S. mansoni infection. Inside the tissue, inflammatory monocytes (IMs) differentiate and polarize in alternatively activated macrophages (AAMs) in a type 2 microenvironment. In this context, we evaluated the role of CD18 on monocytes progenitors and differentiation into AAMs during chronic schistosomiasis. First, we evaluated the monocyte progenitor cells in the bone marrow from wild-type (WT) and CD18low mice. The low expression of CD18 increased the frequency and absolute number of monocyte-macrophage DC progenitor (MDP), while mean fluorescent intensity (MFI) of Ki-67 were reduced on MDP and common monocytes progenitor (cMoP) at 7 weeks post infection (wpi). Next, we evaluated the integrins family in the liver from S. mansoni-infected mice. We showed na increased expression of Itgam when compared to Itgal in the liver at 7 wpi. The frequency of CD11b+ cells also increased at 7 wpi, compared to CD11a+ cells. Moreover, compared to WT mice, CD18low animals reduced the MFI of CD11b and CD11c in IMs. The alternative activation markers, Chil3l3 and Arg1 were reduced in CD18low. Indeed, compared to WT mice, CD18low animals reduced the percentage of CD206+PD-L2+ AAMs in the liver at 7 wpi. In addition , the adoptive transference of IMs to CD18low mice reduces the inflammatory infiltrate in the liver at 7 wpi. Overall, our data indicate CD18 coordinated the monocytes differentiation into AAMs during chronic S. mansoni infection.

P7

Assessment of Immune Responses of Children 3 yo 5 Yrs Vaccinated with Valent10-Pcv and Having Intestinal Nematode Infection

Lynda Allan (The Technical University of Kenya)

Introduction: The integration of pneumococcal conjugate vaccine-valent10 (PCV10) into vaccination program in Kenya has resulted in reduced cases of severe pneumonia diseases in children under 5 years. However, the vaccine efficacy may be limited by confounding factors such as lowered immunity due to infection with intestinal nematodes. The data from this study were collected from 3 to 5 years old children living in Mukuru Kwa Njenga settlement in Nairobi County, Kenya.
Aim: to assess intestinal nematode immune responses of children 3 to 5 years vaccinated with valent10-PCV and having intestinal nematode infection.

Methodology: Children were medically examined for the presence of other infectious diseases by a qualified clinical officer. The intensity of nematode infections was examined in the faecal samples using Kato-Katz technique and expressed as eggs per gram (EPG) of faeces. Serum samples were obtained from blood collected during sampling and used in Flow cytometry to determine expression of IL-2, IL-4, IL-6, IL-10, TNF-α. and IFN-ɣ. ELISA was used to determine secretion of IgG and IgE antibodies.

Results: Ascaris lumbricoides was the only species identified in 34.9% of children having light to moderate infection intensity with mean intensity of 17808EPG. Significant IL-6 levels expressed in children having nematode infection impacted increased secretion of IgG and IgE, thus, an indication that the humoral responses were significantly stimulated. Results indicate suppression of TNF-α expression in the category of children who had nematode infection and were also vaccinated with PCV10, however, a higher expression of IFN-ɣ in the same category implied a negative impact on PCV10 responses. The study also indicated that presence of nematodes and vaccination had a profound influence on the expression of IL-2, however, PCV10 vaccination and intestinal nematode infection did not significantly impact the expression of IL-4 and IL-10 cytokines.

Conclusion: Immune responses due to infection with Ascaris lumbricoides stimulated multiple effector mechanisms influencing induction of cellular responses in PCV10 vaccination.

Recommendation: Investigation of the impact of infection with other parasites on valent-10 pneumonia conjugate vaccine responses in other vulnerable individuals, with focus on other key cytokines.

P9

Antihydatic and immunomodulatory effect of albendazole-pomegranate peel aqueous extract combination on experimental cystic echinococcosis

Moussa Labsi, Imene Soufli, Zine-Charaf Amir, and Chafia Touil-Boukoffa (Laboratory of Cellular and Molecular Biology)

To evaluate the efficacy of combined albendazole (ABZ) - pomegranate peel aqueous extract (PGE) treatment for cystic echinococcosis (CE) in mice, we assess concomitantly in vivo the antihydatic and the immuno-modulatory effects of the combination of ABZ/PGE after 3 months of secondary experimental echinococcosis. Mice were randomly allocated into five groups: ABZ-treated group, PGE-treated group, (ABZ+PGE)-treated group, infested group, and control group. Drugs in diverse treated groups were orally administered daily during CE development for 2 months. After euthanasia, mice were investigated to evaluate the therapeutic efficacy. Cyst development and hepatic damage were macroscopically and histologically analyzed. The hepatic expression of iNOS, TNF-α, NF-κβ, vimentin, and CD68 was examined. Interestingly, the association of ABZ and PGE induced a significant reduction of the rate of hydatid cyst growth inhibition in comparison with the infected or ABZ-treated groups. This effect was related with an amelioration of liver architecture. A significant decrease in iNOS, TNF-α, NF-κβ, vimentin, and CD68 expression was observed in liver tissue of (ABZ+PGE)-treated group compared with infested and ABZ-treated groups. Our finding indicates that the combination of albendazole and PGE treatment is more efficient and suggests its potential preventive value against Echinococcus granulosus infection.

P11

Plasma cell and antibody dynamics during natural models of H. polygyrus infection

Breton Fougere (University of Calgary)

Gastrointestinal helminth infections in livestock are a large economic burden around the world. Despite the prevalence of these infections, few treatments are available that effectively lower parasite burden for a prolonged length of time. Additionally, parasite resistance to drug treatments is rising due to the mass dosing or under dosing of large herds of animals. Naturally resistant animals have been theorized to have higher serum levels of IgG1, resulting in lower parasite burdens. Heligmosomoides polygyrus is a natural helminth

parasite of mice that is used to elucidate parasite clearance mechanisms in vivo. In typical lab studies, mice infected with one large dose of H. polygyrus larvae (bolus) are unable to clear the parasite and are left with high worm burdens in C57Bl/6 mice. However, when animals are given the same number of larvae over an extended period (trickle) they are able to eliminate most or all of the worms. Our group has recently discovered that, despite stark differences in parasite burden, there is no difference in serum IgG1 levels between bolus and trickle animals. However, IgG1 was better able to localize to the host-parasite interface during parasite development in the intestinal tissue. Analysis of plasma cell populations in systemic (BM) and local (MLN) sites showed no difference between the proportions of IgG1+ plasma cells between infected animals at either location; however, the number of IgG1+ plasma cells was higher in the MLN of bolus animals due to an increase in total cell numbers. Overall, our initial results have shown the importance of antibody localization, rather than amount of antibody produced, in the clearance of gastrointestinal helminth infections.

P13

Immunological profile and mechanism of action of γδT cells in Plasmodium ssp. infection

Guilherme Castro (Universidade Federal de Minas Gerais)

The immune response in malaria involves both innate and adaptive immunity cells, and both must overcome the different strategies imposed by the infectious agent. In the context of innate immunity, we can highlight NK, NKT cells and gamma delta T cells (γδ T), all of these molecules are capable of secreting cytokines, producing cytotoxic granules and in theory killing cells infected by Plasmodium. Therefore, our group has been studying the relevance of these cell types, especially γδ T cells in Plasmodium spp infection. In 2021, a study by our group was able to show for the first time the mechanism of action by which a subtype of γδ T lymphocyte is able to lyse and phagocytize both merozoites and infected erythrocytes in an infection caused by P.falciparum. Thus, we can hypothesize that these cells have a similar role in the infection caused by P.vivax, and some preliminary data obtained point out a great importance of γδ T cells in the blood of patients infected with P.vivax. These lymphocytes have a more activated profile in infected individuals, as well as a greater presence of cytotoxic granules, and functional assays have demonstrated the ability of γδ T cells to lyse reticulocytes infected with P.vivax. Altogether our data show the role of γδ T cells in P.falciparum infection and how these cells recognize, kill and phagocytize infected erythrocytes, and some preliminary data support the hypothesis that γδ T cells have a similar role in P.vivax infection.

P15

CCR2 is dispensable for disease resolution but required for restoration of leukocyte homeostasis upon experimental malaria-associated acute respiratory distress syndrome

Emilie Pollenus (Rega Institute for Medical Research, KU Leuven-University of Leuven)

Malaria complications are often lethal, despite efficient killing of Plasmodium parasites with antimalarial drugs. This indicates the need to study the resolution and healing mechanisms involved in the recovery from these complications. Plasmodium berghei NK65-infected C57BL/6 mice develop malaria-associated acute respiratory distress syndrome (MA-ARDS) at 8 days post infection. Antimalarial treatment was started on this day and resulted in the recovery, as measured by the disappearance of the signs of pathology, in >80% of the mice. Therefore, this optimized model represents an asset in the study of mechanisms and leukocyte populations involved in the resolution of MA-ARDS. C-C chemokine receptor type 2 (CCR2) knock-out mice were used to investigate the role of monocytes and macrophages, since these cells are described to play an important role during the resolution of other inflammatory diseases. CCR2 deficiency was associated with significantly lower numbers of inflammatory monocytes in the lungs during infection and resolution and abolished the increase in non-classical monocytes during resolution. Surprisingly, CCR2 was dispensable for the development and the resolution of MA-ARDS, since no effect of the CCR2 knock-out was observed on any of the disease parameters. In contrast, the reappearance of eosinophils and interstitial macrophages during resolution was mitigated in the lungs of CCR2 knock-out mice. In conclusion, CCR2 is required for re-establishing the homeostasis of pulmonary leukocytes during recovery. Furthermore, the resolution of malaria-induced lung pathology is mediated by unknown CCR2-independent mechanisms.

P17

c-MET expression in neutrophils significantly contributes to the pathology of cutaneous leishmaniasis

Katiuska Passelli (University of Lausanne-Department of Biochemistry)

“Leishmania (L.) mexicana is an intracellular protozoan parasite, which causes chronic non-healing cutaneous lesions in humans and mouse with poor parasite control. Neutrophils are the first line of defence against invading pathogens and are rapidly and massively recruited to the site of Leishmania infection. Although neutrophils are professional killers, pathogens including L. mexicana have developed mechanisms to survive in these cells and exploit them as a safe shelter. The presence of neutrophils at the onset of L. mexicana

infection is detrimental, and the factors influencing the recruitment of these cells to the infected skin are therefore of interest in this cutaneous disease. The c-MET tyrosine kinase receptor is crucial for the recruitment of anti-tumoral neutrophils in primary and metastatic tumours, however the role of c-MET expression in neutrophils during parasite infection is unknown. Here, we show that Leishmania infection induces c-MET expression predominantly in neutrophils, with only very low induction in other myeloid cells. Upon infection, mice with genetic deletion of c-MET specifically in neutrophils were able to control better their lesion development and parasite burden, correlating with a reduced frequency of neutrophils infiltration in the chronic lesion. In the same line, systemic administration of the c-MET inhibitor capmatinib in mice with established lesion, promoted a decrease in lesion size associated with a decreased infiltration of neutrophils. Collectively, these data show that L. mexicana-induced c-MET activation in neutrophils contributes to the pathology associated with this disease. These results suggest a potential use for this inhibitor in the control of the cutaneous pathology developing following Leishmania infection.

P19

mTOR mediated immune cell migration leads to immunopathology during Leishmania major infection

Gopinath Venugopal (Department of Microbiology and Immunology, College of Medicine, University of Arkansas for Medical Sciences,)

Leishmania species are the causative agents of cutaneous leishmaniasis, a parasitic disease characterized by the presence of skin lesions. During infection both the parasites and the inflammatory infiltrate contribute to disease. Bulk transcriptomic RNASeq analysis revealed pathways involved in leukocyte trans-endothelial migration, cell adhesion, and chemokine signaling were enhanced in leishmaniasis. In general, immune cell migration is mediated by blood endothelial cells (BECs) binding immune cells and guiding them across the endothelium into the inflamed tissue. However, the mechanisms by which BECs mediate cellular entry into dermal lesions during Leishmania infection is poorly understood. Given immunopathology contributes to disease severity, we sought to investigate the molecular mechanisms responsible for immune cell migration into the tissue. scRNASeq analyses between naïve and L. major-infected mice revealed cellular heterogeneity including distinct resident and recruited cell types in the skin following murine L. major infection. We found BECs from infected skin express elevated transcripts for selectins and adhesion molecules, while concomitantly downregulating transcripts responsible for junctional stability. During infection BECs sense hypoxic conditions in the tissue which is associated with mTOR activation. mTOR target gene expression derived from transcriptomic data reflects mTOR activation in BECs that could possibly support immune cell migration into the dermal lesions. To determine if mTOR signaling contributed to BEC activation, mice were treated with rapamycin, an mTOR inhibitor. Rapamycin treatment decreased BEC selectins and adhesion molecules such as VCAM-1 which reduced the inflammatory infiltrate leading to smaller lesions following L. major infection. Altogether, this comprehensive dataset shows immune cell entry into the dermal lesions is mediated by BEC mTOR signaling during leishmaniasis.

P21

Ephrin B ligands regulate CD8+ T cell function in experimental cerebral malaria

Adesola Olatunde (University of Utah)

Cerebral malaria (CM) is a major fatal complication associated with Plasmodium falciparum infection in humans. Induction of experimental cerebral malaria (ECM) in C57BL/6 mice upon infection with Plasmodium berghei ANKA (PbA) recapitulates some of the clinical symptoms of CM in humans. This model requires antigen-specific CD8+ T cells for disease pathology. However, the molecules that govern T cell migration and trafficking to the brain is incompletely understood. In this study, the role of T cell-expressed EphrinB (EfnB) ligands that bind the Eph B receptor tyrosine kinase subfamily was determined. Using the PbA model of ECM, we observed that activated splenic CD8+ T cells highly express EfnB ligands, particularly in conjunction with expression of IFN-ɣ and TNF-α. Based on published data, we hypothesized that EfnB ligands regulate T cell chemotaxis and recruitment to the brain during ECM. Consistent with this hypothesis more than 90% CD8+ T cells reactive to the P. berghei ANKA GAP50 peptide by tetramer straining expressed EfnB in the brain. Selective deletion of EfnB1 and EfnB2 on T cells (CD4cre+EfnB1/B2fl/fl mice) led to a decrease in the number of activated antigen-specific CD8+ T cells in the brain of EfnB1/B2 deficient mice. We used RNA sequencing to define genes that were differentially expressed by facs-sorted splenic CD8+EfnB+ and CD8+EfnB- cells. Among the genes that were upregulated in CD8+EphB+ cells were genes associated with T cell chemotaxis. Importantly, a decrease in IL-10R expression was observed in CD8+EphB+ cells, which could suggest that these cells populations are pathogenic. However, specific deletion of IL-10R signalling on T cells did not affect EfnB expression. Taken together, our data suggest a potential role for EfnB ligands in T cell trafficking to the brain during ECM.

P23

Leishmania donovani metacyclic promastigotes impair phagosome properties in inflammatory monocytes

Christine Matte (INRS - Centre Armand-Frappier Santé Biotechnologie, Université du Québec)

Protozoan parasites of the Leishmania genus are the etiological agents of leishmaniasis, a debilitating disease with a wide spectrum of clinical symptoms ranging from self-healing ulcers to life-threatening visceral pathologies. These professional vacuolar pathogens are transmitted to mammalian hosts as metacyclic promastigotes by the bite of an infected sandfly and are then rapidly internalized by various phagocyte populations. Inflammatory monocytes are among the first myeloid cells to migrate to infection sites. Recent evidence shows that recruitment of these cells contributes to parasite burden and to the establishment of chronic disease. However, the nature of Leishmania-inflammatory monocyte interactions at the onset of host infection has not been well characterized. Here, we aimed to investigate the impact of Leishmania donovani metacyclic promastigotes on antimicrobial responses within these cells. Our results first showed that L. donovani metacyclic promastigotes rapidly colonized inflammatory monocytes, while Escherichia coli was efficiently cleared in an NADPH oxidase-dependent manner. Using NitroBlue Tetrazolium and confocal immunofluorescence microscopy, we found that parasites inhibited superoxide production at the parasitophorous vacuole (PV) via exclusion of NADPH oxidase subunits gp91phox and p47phox from the PV membrane. Furthermore, using the acidotropic probe LysoTracker, we demonstrated that internalization of L. donovani metacyclic promastigotes produced phagosomes that acidified poorly. Interestingly, while the parasite surface coat virulence glycolipid lipophosphoglycan was involved in preventing PV acidification, impairment of NADPH oxidase assembly was independent of phosphoglycans and of the metalloprotease GP63. Collectively, these observations indicate that permissiveness of inflammatory monocytes to L. donovani may thus be related to the ability of this parasite to impair the microbicidal properties of phagosomes.

Supported by the Canadian Institutes of Health Research

P25

Seroprevalence of Toxoplasmosis and Rubella in Pregnant Women attending Antenatal Clinic in West Africa (Mali and Nigeria)

Mazo Kone, Henrietta Awobode (University of Ibadan)

Background: Toxoplasma gondii and Rubella virus infections are generally asymptomatic but may lead to serious complications during pregnancy. The study evaluation the prevalence of toxoplasmosis and rubella among pregnant women in Ibadan, Nigeria and Bamako, Mali, and also assessed the knowledge, attitude and practice of the women.

Methods: Four hundred and eighty-six (486) pregnant women attending health centres for routine ante-natal care were enrolled voluntarily in a cross-sectional study based on convenience sampling at the Papa & Kadiatou (Pa&Ka) medical laboratory Bamako and five health centres in Ibadan. Antibodies (IgG, IgM) to Toxoplasma gondii and Rubella were assessed by Enzyme immunoassay using chemiluminescence (Elecsys). Questionnaires were administered to obtain demographics, and KAP information of the participants.

Results: The seropositivity for Toxoplasmosis was 42.5%. Both IgG and IgM antibodies were present in 2.2% of the women while 1.8% of the women had only IgM antibodies to Toxoplasma gondii and 36.6% had only IgG antibodies. 96% of women had antibodies to rubella with both IgM and IgG antibodies recording 1.5%. IgG to rubella was recorded in 95.4% of the women against 1.5% for IgM. There is a higher prevalence of IgG and IgM in third trimester women but ToxoIgM was higher in first trimester women.

29.6% of the participants knew that infections could be transmitted to the baby. The source of knowledge about toxoplasmosis was from and from medical personnel (52.5%) and relatives (31.4%). Eating undercooked meat, tasting meat while cooking, and eating raw vegetables were associated with toxoplasmosis (OR: 0.24, 0.52 and 0.50).

Conclusion: There is a high prevalence of Rubella and Toxoplasmosis among pregnant women in the study area. Poor eating choices and low knowledge of the diseases were associated with the high prevalence of infection. This study also revealed a poor knowledge of Toxoplasmosis and Rubella infections.

P27

Cellular context of IL-33 expression dictates impact on anti-helminth immunity

Li-Yin Hung, Yukinori Tanaka, Karl Herbine, Christopher Pastore, Brenal Singh, Annabel Ferguson, Nisha Vora, Bonnie Douglas, Juan Inclan-Rico, Kelly Zullo, Ed Behrens, Tiffany Li Hui Tan, Michael A. Kohanski, Paul Bryce, Cailu Lin, Danielle R. Reed, Breann L. Brown, Noam A. Cohen, and De'Broski R. Herbert (University of Pennsylvania)

Whether interleukin 33 (IL-33) regulates the oscillatory transition between quiescence and inflammation remains unclear. We

demonstrate that IL-33 derived from epithelia drives Type 2 immunity, while IL-33 derived from myeloid antigen presenting cells (APC) suppresses inflammation. Lack of CD11c-restricted IL-33 lowered numbers of intestinal ST2+Foxp3+ Treg, which accelerated anti-helminth immunity. Concurrently, mice with ST2-deficient Treg showed elevated Th2 response after hookworm infection. We further identified an IL-33 induced pore-forming protein, Perforin-2, on DC that was essential for spontaneous IL-33 release from CD103+ DC and inhibition of Perforin-2 activity blocked the proliferative expansion of ST2+Foxp3+ Treg. This work indicates that APC can utilize Perforin-2 as a novel plasma membrane conduit for IL-33 delivery to promote mucosal immunoregulation.

P29

Characterization of Vivaxin, a novel bloodstream-stage, species-specific, cell-surface family as potential vaccine candidates against the livestock parasite Trypanosoma vivax

Alessandra Romero-Ramirez (Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool)

Trypanosoma vivax is a major animal pathogen causing African Animal Trypanosomiasis (AAT) or Nagana affecting livestock across Africa and South America. No vaccine is available for AAT due to antigenic variation of the Variant Surface Glycoprotein (VSG) coating the parasite surface, leading to an effective immune evasion. However, the T. vivax genome contains diverse species-specific genes that encode cell-surface proteins (TvCSP) expressed in the bloodstream stage demonstrating that the surface coat consists of invariant proteins besides the VSG, which might be vaccination targets. Using a reverse vaccinology approach, we examined these TvCSP and their utility as vaccines against the parasite. In silico sequence analysis of TvCSP indicates that most TvCSP are transmembrane proteins, present in diverse clinical isolates and containing minimal polymorphism. The identification of immunogenic linear B-cell epitopes based on a customized peptide microarray reveals one protein family (FamX) to be the most immunogenic in natural infections (besides VSG), and four FamX proteins were successfully expressed in recombinant form. FamX proteins are used to immunize BALB/c mice with multiple adjuvants prior to parasite challenge to explore the resultant immune response and the protective properties of vaccination. Immunization stimulated high levels of pro-inflammatory cytokines indicating that FamX proteins stimulated a mixed Th1/Th2-type immune response, and one antigen (AJ6), co-administrated with a Quil-A adjuvant, induced partial protection with 60% efficacy in mice. AJ6 was also localized to the cell-surface based on immuno-fluorescence microscopy. This is the first report of a novel protein component of the T. vivax surface coat that is invariant and immunogenic, which offers promise of an effective vaccine for animal African trypanosomiasis. The identification of potential vaccine candidates based on invariant surface proteins can revolutionize the fight against AAT bringing unprecedented benefits to animal health and agricultural productivity across the world

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P3

Humoral and Cell-Mediated Immune Response Validation in Calves after a Live Attenuated Vaccine of Babesia bigemina

Umber Rauf (University of Veterinary and Animal Sciences)

The current vaccines to control bovine Babesia bigemina(B. bigemina) infection are not fully protective and vaccination failures incur heavy losses to the cattle industry around the world. Using modified micro-aerophilous stationary phase, we developed a culture-derived attenuated live vaccine against B. bigemina and tested a single subcutaneous inoculation of 2 × 108 infected erythrocytes in calves. The protection was measured after a lethal intravenous challenge with 5 × 108 virulent calf-derived B. bigemina. Our results demonstrated that a single shot of attenuated vaccine was capable of inducing robust humoral and cell-mediated immune responses in calves. We found a significant increase in the IgG antibody titers post-challenge and a strong proliferation of both CD4+ and CD8+ T cells contributing towards the protection. Our vaccine provided complete protection and parasitic clearance, which was followed for more than 100 days post-challenge. This immunity against babesiosis was directly linked to strong humoral responses; however, the parasitic clearance was attributed to significant T cells effector responses in vaccinated calves as compared to the infected control calves. We anticipate that these results will be helpful in the development of more efficient culture-derived vaccines against Babesia infections, thus reducing significant global economic losses to farmers and the cattle industry.

P31

Causes and consequences of thrombocytopenia in experimental murine visceral leishmaniasis

Gulab Fatima RANI (PhD student)

Visceral leishmaniasis (VL) is a neglected tropical parasitic disease caused by the protozoan parasites Leishmania donovani and L. infantum. VL manifests as fever, weight-loss, hepatosplenomegaly, immune dysregulation and hematological complications.

Thrombocytopenia is a dominant hematological feature of VL, both in humans and experimental models but the mechanisms behind this infection-driven thrombocytopenia remain poorly understood. Using the C57BL/6 model of experimental VL, we demonstrate a progressive decrease in platelets from d14 post-infection culminating in severe thrombocytopenia by d28. Plasma thrombopoietin levels and cytoplasmic demarcation membranes in bone marrow megakaryocytes were reduced in infected mice suggestive of defective platelet production. In addition, we identified significant increases in platelet clearance. Splenectomised infected mice were protected from thrombocytopenia compared to sham-operated infected mice and had a greater response to exogenous thrombopoietin. Furthermore, infection led to higher levels of platelet opsonisation and desialylation, both associated with platelet clearance in spleen and liver respectively. This profound thrombocytopenia was rapidly reversed following single dose AmBisome® treatment, along with multiple other markers associated with immune activation (including restoration of tissue microarchitecture and reduced macrophage iNOS expression). Moreover, mice cured of VL showed comparable albeit delayed clinical responses (including peak parasitemia, anemia and thrombocytopenia with reduced plasma thrombopoietin) to subsequent malaria. Our findings demonstrate that the mechanisms underpinning thrombocytopenia in experimental VL are multifactorial and reversible, with no obvious residual damage to the bone marrow microenvironment. We have also demonstrated that in the mouse model a previously drug-cured episode of VL has limited impact on the subsequent development of malaria. However, the question of whether more clinically severe or protracted VL can impact the progression of subsequent infections such as malaria deserves attention in alternate models or through longitudinal population studies in humans.

P33 – Withdrawn

P35

Thermoneutrality: an experimental condition to consider in study of parasitic infection models

Fiorella Vialard (Research Institute of the McGill University Health Centre)

Although most studies using mouse models for disease are conducted at sub-optimal housing temperature (ST), experiments performed at thermoneutral temperature (TT) revealed an altered immune response to pathogens. However, little is known about the effect of temperature on parasitic disease models. Specifically, no studies have been conducted at TT on the malarial severe disease model or Leishmania infection.

We hypothesized that (1) the innate immune response to malarial hemozoin (HZ) or Leishmania major injection in a peritonitis model and (2) disease progression in mice infected with Plasmodium berghei ANKA (PbA) would be different at TT compared to ST.

To test our hypotheses, C57BL6 female mice were individually housed for 3 weeks at TT (28 – 31°C) or ST (20 – 22 °C). In one study, mice were injected intraperitoneally with HZ or L. major promastigotes at both temperatures. Immune cells isolated from the peritoneal cavity (PEC) fluid 6 hours after injection were counted and sub-typed using microscopy. Inflammatory cytokine levels and extracellular vesicle (EV) profiles were also determined from the PEC. L. major injection resulted in lower levels of immune cell recruitment and EV release driven by neutrophils in the PEC of mice housed at TT compared to ST. We also observed a trend of higher phagocytic activity in mice housed at TT and different cytokine level profiles at TT compared to ST.

In another study, mice were injected with 104 PbA-infected RBCs. Parasitemia, weight, temperatures and clinical scores were monitored until end-point, when blood, lung, spleen and liver were collected. We observed a trend of delayed onset of disease in animals housed at TT compared to ST and different levels of serum cytokines in animals housed at different temperatures. Our findings provide evidence that housing temperature can influence the immune response to parasitic agents in murine models of disease.

P37

Nippostrongylous brasiliensis transcriptionally adapts to host immune status

Annabel Ferguson (University of Pennsylvania)

Soil-transmitted helminth infections are responsible for millions of disability associated life years (DALYs) annually in people across the globe. Despite the initial promising efficacy of anthelminthic drugs in reducing worm burdens, emerging evidence indicates that drug resistance develops over time, which highlights the need to understand the biological underpinnings of parasite adaptation. Hookworms can dramatically modulate their morphology and behavior depending on life-cycle stage and/or environment. This work addressed whether the rat hookworm N. brasiliensis can modulate its transcriptome depending on host immune status. We hypothesized that transcriptomic differences in adult worms would occur in immunodeficient hosts as compared to wild-type hosts and this change would be inherited by subsequent parasite generations. The N. brasiliensis life cycle was sequentially passaged for 6 generations in RAG1 KO mice

lacking T and B cells, to determine if adaptive immunity was a key driver of helminth-intrinsic transcript expression. Bulk RNA-sequencing was performed on samples containing 10 male and 10 female adult stage parasites isolated on day 7 post infection from cohorts of WT and RAG1 KO mice, with 2 samples collected per host. The data shows that RAG-1 adapted worms had ~40 significantly down-regulated transcripts and 15 significantly up-regulated transcripts compared to WT passaged worms. Interestingly, among the down-regulated transcripts are several uncharacterized genes coding for CAP superfamily proteins, among which are venom allergen-like proteins shown to elicit a strong allergic Type 2 inflammatory responses. Using functional assays to assess worm survival and fecundity in WT hosts, data show that RAG1-KO-adapted parasites demonstrated greater virulence than the WT-passaged worms. These results may indicate that N. brasiliensis becomes less immunogenic in hosts lacking adaptive immunity, which results in greater parasite burden. Overall, this study paves the road for further investigation into how parasitic nematodes adapt to distinct arms of host protective Type 2 immunity.

P39

Studying lymphocyte responses during blood-stage Plasmodium infection using spatial transcriptomics

Cameron Williams (Peter Doherty Institute, Melbourne, Australia)

Lymphocyte differentiation is a key process in mounting effective immune responses. In recent years, this process has been mapped with single-cell RNA sequencing (scRNA-seq) technologies that allow unbiased, whole-transcriptome assessments without requiring specialist reagents such as monoclonal antibodies. However, scRNA-seq does not assess cells within their normal tissue context. We explored a spatial transcriptomics technique, Slide-seq2, for studying the effect of cell positioning and interactions on lymphocyte differentiation in the spleen, before and 7 days after infection of C57BL/6J mice with P. chabaudi chabaudi AS. We first hypothesized that immune cells and splenic micro anatomical structures are identifiable from transcriptomes alone. Consistent with this, having grouped similar transcriptomes via unbiased clustering, we found regions corresponding to red pulp, B cell follicles, and T cell zones. This analysis also revealed structural changes induced by infection, including B cell follicle enlargement. We next hypothesized that Slide-seq2 could be used to map detailed lymphocytic responses. To address this, we generated a parallel, high-depth, droplet-based scRNA-seq reference dataset of splenic immune cells and mapped this reference to the spatial transcriptomics dataset via Robust Cell Type Decomposition (RCTD). We found that while Slide-seq2 transcriptomes frequently contained mixtures of cell types, competing influences could be deconvoluted, for example as pre-GC B cells, plasmablasts (PBs), CD4+, and CD8+ T cells, thus allowing inference of their locations. Interestingly, we observed unique bystander-like transcriptomes among non-PB, non-pre-GC B cells at day 7, suggesting utility of our approach for discovery. In ongoing analyses, e.g., with Tangram, we aim to discover novel molecules controlling intra- and extrafollicular interactions between B cells and CD4+ T cells.

P41

Using CRISPR/Cas9 in primary natural killer cells to study antibody-dependent cellular cytotoxicity against malaria-infected red blood cells

James Dahlvang (University of Minnesota, Department of Medicine, Center for Immunology, Division of Infectious Disease and International Medicine)

Increasing evidence suggests that natural killer (NK) cells play a protective role in the immune response to blood-stage malaria. Having a higher proportion of adaptive NK cells—defined as NK cells that lack both the Fc receptor gamma chain (Fcer1g) and transcription factor PLZF—correlated with reduced parasitemia and protection from malaria symptoms in a non-severe malaria cohort. We hypothesize that adaptive NK cells are protective due to their enhanced antibody-dependent cellular cytotoxicity (ADCC) function. However, we still do not know why adaptive NK cells have enhanced ADCC function or how they kill malaria-infected red blood cells (iRBCs). While NK cells that lack Fcer1g show enhanced ADCC function, it is unknown whether the loss of Fcer1g enhances function per se or if it is only a marker of adaptive NK cells. To test this, we developed a CRISPR/Cas9 method for primary NK cells. We ablated genes including, but not limited to, Fcer1g and CD247—the gene encoding for the CD3zetachain—to test how the loss of these genes affected ADCC function. Results from these experiments will be shown. We also used our CRISPR/Cas9 technique to better understand how NK cells kill iRBCs. NK cells release lytic granules containing perforin, granzymes, and granulysin in response to opsonized cancer cells. This triggers the apoptosis pathway and results in programmed cell death. Mammalian red blood cells (RBCs) are anucleated and lack mitochondria. This makes RBC apoptosis impossible, meaning that granzyme B/granulysin should not be able to induce RBC death. However, iRBCs contain a nucleus and mitochondria from Plasmodium, meaning granzyme B/granulysin may be able to induce cross-species apoptosis on the parasite. To better understand the killing mechanism of iRBCs, we ablated the genes encoding for lytic proteins such as granzyme B and granulysin and evaluated how well the mutant NK cells inhibited parasite growth. Results from these experiments will also be shown.

P43

T-bet expression in atypical memory B cells in a rodent malaria model

Lauren Sullivan (The Francis Crick Institute)

Atypical memory B cells (aMBC) have been observed in the peripheral blood of individuals infected with Plasmodium falciparum malaria, as well other chronic human infections, including HIV and TB. There is growing evidence to suggest these aMBC are part of a normal immune response to chronic infection. However, it is difficult to determine their role and function in human infections where only peripheral blood is available for analysis.

We have shown in a mouse model of malaria, C57BL/6 mice infected with P. chabaudi AS, that aMBC (CD11c+CD11b+IgDlo) are activated in chronic infection and following protein immunisation. This will allow us to investigate the origin and function of aMBCs. Classical memory B cells (cMBCs) undergo somatic hypermutation and class switching via the germinal-centre reaction, resulting in the clonal expansion of B cells producing higher affinity antibodies. cMBCs persist after clearance of infection and are reactivated in secondary responses. The kinetics of aMBC generation and maintenance, their ability to undergo germinal centre-reactions and/or secrete high affinity antibodies is not known.

Here we describe the surface phenotype and expression of the transcription factor T-bet in mouse aMBC and cMBC in a P. chabaudi infection. We generated mixed-bone marrow chimeric mice, in which the B cell compartment contained 1-3% of B cells that carry a “knock-in” immunoglobulin heavy chain, which together with endogenous light chains, recognise the C-terminus of P. chabaudi merozoite surface protein 1. The numbers of both aMBCs and cMBCs in the spleen were determined throughout the acute and chronic infection.

Atypical memory B cells are already generated in the acute stage of a mosquito-transmitted blood-stage P. chabaudi infection, alongside cMBCs. Unlike cMBCs, they decline during the chronic phase of infection, and are not detectable after immunity-meditated parasite clearance, suggesting that these cells are not memory cells in the functional sense. They may be a response to persistent antigenic stimulation rather than the cause of the chronic infection.

P45

Evaluation of Plasmodium vivax antigens as vaccine candidate to Plasmodium spp.

Luna de Lacerda (Oswaldo Cruz Foundation)

The bottleneck of the malaria vaccine is the challenge to find multi-stage antigens conserved in different Plasmodium spp. Most malaria vaccines focus on antibody induction to the pre-erythrocytic stage, even though the sterile vaccine protection induced by attenuated sporozoites is directly related to cytotoxic T cells (CTLs). Our group lately demonstrated that P. vivax (Pv)-infected reticulocytes can activate CTLs, in an antigen-dependent-manner, describing a protective mechanism against blood-stage parasites. In unpublished data, we perform the immunopeptidomics analysis aiming to identify HLA-I-associated peptides of Pv-infected reticulocytes. It was assessed that 60% of the eluted peptides are derived from ‘housekeeping’ proteins, which are conserved in Plasmodium cross-stage and cross-species. Afterward, we selected 50 peptides and their source proteins to be studied, for the first time, as a potential malaria vaccine candidate. P. yoelii (Py) infection is an excellent experimental model for investigating malaria immune response. First, we tested the 50 peptides in an anti-IFNɣ ELISPOT assay with Py-infected mice splenocytes. Then, we produce three recombinant proteins (L30, S25, and S30) in the E. coli expression system for immunization protocols. Mice were immunized three times with each protein associated with Alum + CpG as an immunological adjuvant. Twenty-one days after the last boost, mice were challenged with 106 PyX17NL-infected red blood cells, and the parasitemia was monitored for 30 days. We identify 23 of the 50 Pv peptides were immunogenic in the acute infection, and 12 remain in the convalescent phase, suggesting an immune-memory response. Regarding the immunization assessment, we identify that 2 of 3 tested proteins can induce antigen-specific IgG Total and IgG2c levels but not IgG1, additionally, to decrease in 50-80% the parasitemia compared to the control group. Therefore, we identify newly Pv antigens that can induce a protective response to Py infection, indicating cross-species protection and a potential vaccine candidate.

P47

Analysis and characterization of metabolites in excretory-secretory products secreted by gastrointestinal nematodes as immunomodulators of inflammatory bowel disease

Elizabeth Siciliani (Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, QC, Canada)

Infection with gastrointestinal (GI) nematodes including Heligmosomoides polygyrus bakeri (Hpb), Trichuris suis, and Ascaris suum modulates immune responses to unrelated pathogens and bystander antigens. This is thought to be mediated by excretory-secretory products (ESP) released by these parasites. Although immunomodulatory proteins and miRNAs have been identified in ESP, little is known about the

metabolites released by parasitic worms. To determine if metabolites in ESP have immunomodulatory effects, we prepared conditioned media from Hpb, T. suis, and A. suum and isolated polar (P) and non-polar (NP) metabolites. NP but not P metabolites in ESP produced by these parasites suppressed LPS-stimulated TNFɣ secretion and enhanced IL-10 secretion by bone marrow-derived macrophages (BMDM) from C57BL/6 mice. Increased fluorescence of the dye Alamar Blue, a redox indicator, indicated important metabolic changes in NP metabolite-treated BMDM. In addition, BM precursors incubated with A. suum NP metabolites during differentiation had fewer BMDM-like cells. To identify the metabolite(s) responsible for the modulatory effects of ESP, A. suum ESP was analysed by LC-MS/MS, separated by RP-HPLC, and the bioactivity of the fractions assessed on LPS-stimulated BMDM. RP-HPLC fractions that suppressed TNFα secretion coincided with UV/Vis peaks identified in previous analyses of T. suis. We are in the process of performing LC-MS on NP metabolites. The therapeutic effects of AsNP25 were investigated in DSS-colitic C57BL/6 mice. Treatment with AsNP25 resulted in significantly higher colon length, lower histopathology score, and decreased TNFα mRNA in colon tissue compared to PBS control mice. Together, these data suggest NP metabolites in ESP secreted by parasitic worms modulate BMDM in vitro and ameliorate disease in DSS-induced colitis in mice.

P49

Lumican is required for the control of parasitemia in malaria

Margaux Sica (NYU School of Medicine)

Malaria still causes approximately 400,000 deaths annually in the developing world, a majority of which are children under the age of 5. Using a mouse model for malaria, we have determined that Lumican, an extracellular matrix protein that belongs to the small leucine-rich family of keratan sulfate proteoglycans, plays an essential role in the control of parasite growth. Wild-type mice are able to recover from a murine nonlethal Plasmodium yoelii malaria infection and continue to live a normal life. On the other hand, mutant mice deficient in Lumican cannot control parasite levels and succumb to death. While innate and adaptive immunity are both important to fight a malaria infection, we observe that levels of parasitemia diverge between wild-type and Lumican-deficient mice 12 days after P. yoelii infection, suggesting that Lumican contributes to the adaptive immune response to infection. We have observed that Lumican-deficient mice produce lower levels of IgG2a to P. yoelii and present lower levels of F4/80+ CD11b+ macrophages, which are both important mechanisms in the control of parasitemia. Since IFN-gamma levels are decreased in Lumican-deficient mice, and this cytokine is required for IgG2a switch and for macrophage activation, we hypothesize that IFN-gamma plays an important role in the lack of parasitemia control of Lumican-deficient mice. Taken together, these results indicate a fundamental role of Lumican in the immune response to malaria.

P51

Investigating the role of mast cell-erythrocyte progenitors in anemia recovery

Hannah Federman (Rutgers New Jersey Medical School)

Helminth infections affect more than 2 billion people of all age groups throughout the undeveloped world, leading to distinct morbidities and increased economic hardships. Although anemia is often cited as a major helminth-associated morbidity, it remains poorly understood, challenging to diagnose, and ultimately difficult to treat. Our recent work has identified a unique population of bone marrow-derived progenitor cells that are defined by their expression of c-Kit, integrin β7, and the metabolic enzyme carbonic anhydrase 1 (Car1). Further, our studies show that these unique cells are bipotent, with the capacity to differentiate into either mast cells or erythrocytes. Moreover, our data demonstrate that these progenitors are mobilized and simultaneously support the protective mast cell response following a Trichinella spiralis infection and the erythropoietic response to the resultant infection-induced anemia. Despite these advances, the ability of these cells to respond to anemia-induced stimuli in the absence of protective mast cell responses remains to be defined. Therefore, we sought to determine the role these cells play in the recovery from both chemically-induced anemia and anemia induced by the hookworm Nippostrongylus brasiliensis, a parasite that is expelled independently of mast cell responses. Our data suggest that Car1+ mast cell-erythrocyte progenitors play important roles in anemia recovery no matter the anemia's etiology. Further these data suggest that while Car1+ cells are bipotent in nature, their mast cell and erythropoiesis capacity can be uncoupled depending on the developmental signals they receive. Thus, we believe that this work could lead to a bridge between the differentiation pathways of mast cells and erythrocytes and, in turn, more effective treatments for not only helminth-induced anemia, but anemias of myriad sources.

P53

Malnutrition disrupts mucosal immunity and protective immune responses during visceral leishmaniasis

Lais Sacramento (University of Pennsylvania - Department of Pathobiology)

Protein malnutrition is a risk factor for developing visceral leishmaniasis (VL) due to defective immune responses that culminate in early visceralization. The intestine is a target of both malnutrition and infection, but the immunological events that occur there during infection

are unknown. We postulated that malnutrition exacerbates VL due to defective immunological mechanisms to control parasite replication in the spleen and liver, and by modifying intestinal mucosal immunity. To study the effect of malnutrition on immune response in chronic VL we used a polynutrient deficiency (deficient protein, energy, zinc, and iron) diet which mimics moderate human malnutrition followed by Leishmania infantum infection. The polynutrient deficient diet leads to growth stunting, reduced mass, and cellularity of the spleen, liver, and mesenteric lymph node (MLN). Malnourished-infected mice were more susceptible to infection, harboring more parasites in the spleen and liver. Flow cytometry analysis revealed a reduced number of T lymphocytes, and reduced production of IFN-ɣ by T cells in malnourished-infected mice. In the MLN, L. infantum infection induced the production of IFN-ɣ and TNF, while malnourished-infected mice exhibited a significant decrease in the IFN-ɣ production by T cells. Also, malnourished-infected mice presented a reduction in the IL-17 and IL-22 production by CD4+ cells, which are critical components for intestinal integrity and homeostasis. Together, malnutrition causes a defective IFN-ɣ-mediated response and promotes dysfunctional mucosal immunity which increases susceptibility in VL.

P55

Myeloid-derived IL-33 limits expansion of cutaneous γδ T cell responses during Schistosoma mansoni infection.

Juan M. Inclan-Rico (Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania.)

The skin is a critical structural and immunological barrier to 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 that regulates skin homeostasis and immunity against S. mansoni cercariae. Our data show that selective deficiency in CD11c-derived IL-33 augmented IL-17 responses under basal conditions and led to enhanced resistance to cercarial skin penetration relative to controls. Reduced skin penetration in CD11c-specific IL-33 deficient mice was also associated with elevated numbers of dermal γδ T cell subsets and diminished frequency of dermal GATA3+ ST2+ T regulatory cells, suggesting that myeloid-derived IL-33 prevents spontaneous cutaneous inflammation. Furthermore, loss of CD11c-derived IL-33 led to diminished adult worm burdens and egg deposition in the liver and intestine by 8 weeks post-infection relative to controls. Collectively, these results highlight a previously unrecognized circuit involving skin-resident DCs, γδ T cells and GATA3+ ST2+ T regulatory cells that together maintain cutaneous homeostasis, but that can be exploited by skin-penetrating helminths to promote host infection.

P57

An exposure biomarker specific to Phlebotomus argentipes bites towards sustainable vector control in the Indian sub-continent

Eva Iniguez (Laboratory of Malaria and Vector Research NIH/NIAID)

Visceral leishmaniasis is a vector-borne neglected disease that represents a serious global public health concern, causing more than 40,000 deaths annually with a disproportionate burden in the Indian sub-continent. In the Indian sub-continent, visceral leishmaniasis is transmitted exclusively via the bite of a Leishmania donovani-infected female sand fly, Phlebotomus argentipes; while the only other man-biting sand fly species present in the region, Phlebotomus papatasi, lacks vectorial capacity. Since there is no human vaccine for visceral leishmaniasis, measures to control the disease depend on early case-detection, treatment, and reduction in transmission through vector control measures. Current epidemiological surveillance tools are limited to sand fly capture by light traps to monitor sand fly prevalence. Therefore, tools to assess actual exposure to sand fly bites are urgently needed. Humans living in endemic areas are constantly bitten by sand flies and develop a strong antibody response to specific sand fly salivary proteins. Here, we identified two immunodominant and species-specific proteins from saliva the sand fly Ph. argentipes for use as biomarkers of vector exposure. Herein, we provide data on the immunodominance and specificity of recombinant Ph. argentipes salivary proteins, rPaA and rPagB, and validate their use in combination as a potential biomarker of exposure to Ph. argentipes bites from individuals living in visceral leishmaniasis-endemic villages in Bihar, India. Finding a marker of vector exposure to Ph. argentipes will provide a powerful, much-needed tool for efficient implementation of vector control interventions, assessment of risk of acquiring the disease and may possibly contribute to prevention of visceral leishmaniasis outbreaks in the Indian sub-continent.

P59

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

Gaurav & Gupta (NIIT University, India)

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.

P61

Gut microbiota composition modulates the magnitude and quality of germinal centers during Plasmodium infections

Rafael Polidoro (Indiana University School of Medicine)

The gut microbiota has been shown to play a role in both human and rodent Plasmodium infections. In mice, gut microbiota composition can modulate the severity of malaria, leading to differences in blood stage parasite burden and the duration of infection. Yet, the mechanism by which gut microbiota impacts the severity of malaria remains unknown. While humoral immunity is critical in mediating clearance of Plasmodium blood stage infections, the interplay between gut microbiota, parasite burden, and antibody production is not well characterized. This prompted the hypothesis that mice with lower parasite burden will exhibit better germinal center responses. In support of this hypothesis, there is a gut microbiota dependent increase in numbers of germinal center B cells and parasite-specific antibody titers that is inversely correlated with parasite burdens. These mice with low parasite burden also demonstrate better maintenance of germinal center structure and a more targeted antibody response. Enhanced humoral immunity during the primary Plasmodium infection also impacted memory, as mice with low parasite burden were protected against challenge with a heterologous, lethal Plasmodium species. These results provide mechanistic insight on impact of the gut microbiota on extra-gastrointestinal tract germinal center response and will be important in the development optimal treatments for malaria.

P63

Toxoplasma gondii strains can be differently recognized in chronically infected pregnant women from South of Brazil.

Ize A. Bittencourt (Carlos Chagas Institute, Fiocruz, Paraná)

About a third of the human population is chronically infected with toxoplasmosis and most of infections are asymptomatic. However, severe toxoplasmosis might occur even in immune competent individuals. One of the factors that contributes to the disease severity is the genetic variability of Toxoplasma gondii strains. Genotyping methods can be applied to identify circulating T. gondii strains but those methods depend on the availability of parasite DNA. An alternative approach is the use of serotyping methods, which are based on the presence of long-lasting specific antibodies to T. gondii polymorphic epitopes. However, improvement in serotyping methods is still necessary, especially in regions where T. gondii has high strains variability. Here, we established a flow cytometry protocol using fluorescent parasites cultured in vitro to analyze the reactivity of antibodies from T. gondii chronically infected pregnant women from the city of Curitiba, South of Brazil. The use of whole fluorescent parasite increased the accuracy of the technique and allowed to distinguish T. gondii strains in comparison with the IgG reactivity to GRA peptides measured by ELISA. Based on the values of antibody reactivity of pregnant patients from Curitiba, we observed the genetic variability of the strains circulating in this population. We believe that flow cytometry is a promising approach for serotype analysis and its improvement regarding the serotyping of strain diversity will bring new insights to the disease epidemiology.

P65

Transcriptomes of Neutrophils from Individuals with Cutaneous Leishmaniasis, Subclinical L. braziliensis Infection and Healthy Controls in Northeast Brazil.

Jacilara Conceicao (Postdoctoral Research Scholar)

Individuals with Leishmania braziliensis subclinical (SC) infection are characterized by (1) a positive leishmania skin test and/or high peripheral blood mononuclear cell (PBMC) production of IFN-ɣ when stimulated to leishmania antigen, and (2) the absence of current or past clinical manifestations of cutaneous leishmaniasis (CL). Preliminary data showed that SC individuals' neutrophils (PMNs) may contribute to disease prevention whereas these cells promote immunopathogenesis during CL. We addressed the hypothesis that transcriptional profiles would yield a more complete view of metabolic and inflammatory status of neutrophils from both groups. Anti-CD15 ultra-purified neutrophils were separated from peripheral blood of SC individuals, CL patients or healthy controls (HCs) yielded 98.9% ± 1.14, 99.1% ± 0.55 or 100% ± 0 pure PMNs (mean ± SD), respectively. RNA was extracted, subjected to RNA-seq, and analyzed using Kallisto and RankProd. Data analysis showed upregulation of CASP1 [fold change (FC)= 2.24, corrected P<0.001 (ANOVA)], BTN3A1 (FC= 2.26, P<0.001) and BTN3A3 (FC= 2.47, P<0.001) in CL PMNs compared to SC PMNs. For validation, RT-qPCR of the same samples demonstrated that CL PMNs trend toward higher expression of CASP1, BTN3A1 and BTN3A3 compared to PMNs from SC or HC individuals. CASP1 plays a central role in inflammasome activation and has been shown to be involved in immunopathology of CL. Other studies have shown that BTN3A1 and BTN3A3 modulate gamma delta T cell (gdT) activation via the TCR. These data underscore the importance of PMNs in the inflammasome mediated pathogenic response in CL patients, and the lack of PMN transcripts associated with inflammation in SC subject PMNs. Also implicated is a role for gdT cells, a cell type that was already documented to be involved in PMN recruitment in murine CL. These data suggest that PMN plasticity is invoked to mediate the pathogenesis of L. braziliensis infection.

P67

CD40 Drives Protective T Cell Responses Against Cryposporidium in a Novel Mouse Model

Keenan O'Dea (University of Pennsylvania)

The apicomplexan intestinal parasite Cryptosporidium infects intestinal epithelial cells (IECs) and is a leading cause of life-threatening disease in infants, toddlers, and immunocompromised patients. No vaccine against Cryptosporidium exists, and current treatments are ineffective in immunocompromised individuals. A prominent immunodeficiency associated with susceptibility to Cryptosporidium is hyper-IgM syndrome, which is caused by defects in CD40 signaling. In a novel murine model of Cryptosporidiosis, I have shown that disease progression in mice deficient in CD154 (CD40L) closely mimics human disease, and that complementation of CD40L restores protection. Therefore, characterizing CD40-mediated immune responses against Cryptosporidium may enhance progress toward effective therapy in at-risk individuals. CD40 is a trimeric receptor belonging to the tumor necrosis factor receptor (TNFr) superfamily, which, when activated via engagement by CD40L, can induce a multiplicity of downstream effects. CD40 engagement on dendritic cells (DCs) promotes IL-12 production that induces differentiation and proliferation of T helper type 1 (Th1) cells and their production of interferon-ɣ (IFN-ɣ), which is known to be essential for control and clearance of Cryptosporidium infection. Data from our group suggest a defect in T cell responses and IFN-ɣ production in CD40-/- mice infected with Cryptosporidium. Therefore, we conclude that CD40 mediates T cell responses against Cryptosporidium.

P69

Development of a system for tracking CD4+ T cell responses to Cryptosporidium

Ian Cohn (University of Pennsylvania)

Protective immunity to the intestinal parasite Cryptosporidium requires CD4+ T cells. Analyses of CD4+ responses have been limited to bulk populations, as no MHCII-restricted antigens are known and most of the CD4+ T cells in the gut possess an "activated but resting" phenotype," preventing the use of activation markers for identifying cells responding to the parasite. We have developed a novel system for tracking Cryptosporidium-specific CD4+ T cells. Using CRISPR/Cas9, we have generated transgenic Cryptosporidium parvum expressing the 2W1S and gp61 model antigens tagged onto the secreted protein MEDLE2. We have measured parasite-specific CD4+ T cells in the draining mesenteric lymph node, the Peyer's patch, the small intestine lamina propria, and the epithelium in infected mice using both of these antigens. We observed expanded populations of 2W1S-specific CD4+ T cells using MHCII-tetramers in all of these compartments, and SMARTA T cells after adoptive transfer from TCR-transgenic mice when using the gp61 model antigen. This tool allows for investigation of the antigen presenting cells and location(s) responsible for CD4+ T cell priming, as well as the phenotype of the CD4+ T cells responding to Cryptosporidium.

Tuesday, April 13

P2 – Withdrawn

P4

Concomitant infection of Leishmania donovani and Plasmodium berghei reduces disease severity in BALB/c mice

Rebeccah Ayako (Institute of Primate Research)

Malaria and visceral leishmaniasis coexist in the same geographical regions. However, dual co-infection with parasites causing these diseases and their impact on public health is poorly documented. Interactions between these parasites may play a role in disease outcome. The present study set out to evaluate the clinical, parasitological outcome and humoral immune responses following Leishmania donovani and Plasmodium berghei co-infection in BALB/c mice. Mice were divided into four groups; L. donovani- only, L. donovani- P. berghei, P. berghei- only and naïve. Body weight, L. donovani parasite load, P. berghei parasitemia and total IgG responses were determined. Spleen, liver and brain tissues were obtained for histological analysis. Four mice from each group were used for monitoring the survival rate. Results indicated significant differences in body weight ($p<0.0001$), L. donovani parasite load ($p< 0.0001$), L. donovani IgG ($p< 0.0001$), P. berghei parasitemia ($p= 0.0345, 0.0222$) and P. berghei IgG ($p= 0.02, 0.002$) in both single and dual infections respectively. There was no correlation between L. donovani parasite load and IgG responses in single or dual infections, while a positive relationship of P. berghei parasitemia and IgG responses was observed in the dual infected group only. Histological analysis revealed the enlargement of red and white pulps, formation of granulomas in the spleen and vascular degeneration in the liver which were more pronounced in single infected groups. Leukocyte sequestration and microhemorrhages were more defined in the P. berghei- group. Plasmodium berghei had the highest mortality rate compared to L. donovani- only and L. donovani- P. berghei infected mice groups. We conclude that L. donovani and Plasmodium co-infection reduces disease severity and could possibly interfere with serological and parasitological disease diagnosis. Therefore, the study recommends policymakers to employ early detection as a new way of diagnosis to reduce mortality and morbidity regions of co-endemicity.

P6

Cryptosporidiosis in Infants in Bangladesh results in immunity to diarrhea but not infection nor infection-associated growth faltering.

Mamun Kabir (University of Virginia)

We conducted a longitudinal study of cryptosporidiosis from birth to three years of age in an urban slum of Dhaka Bangladesh. Fecal DNA was extracted from monthly surveillance samples and diarrheal stool samples collected from 392 infants from birth to three years. One hundred and twenty one (31%) of children experienced a single infection while two hundred and twenty two (57%) had multiple Cryptosporidium infections. Repeat infections had a lower burden of parasites in the stool (Cq slope = -1.85; $p<0.0001$) and were more likely to result in sub-clinical disease (Chi square test for trend; $p=0.01$). Repeat infections were associated with the development of growth faltering (Pearson correlation = -0.18; $p=0.0004$). Fecal IgA antibodies against the Cryptosporidium CP23 sporozoite protein were measured at one year in age (mean value 1.913 ± 1.358 [0.022- 4.342]) permitting these infants to be sub-grouped depending on whether they had a high or low IgA response (Group One: High-IgA responders; Group Two:Low-IgA). Even in this restricted subgroup (children who remained in the study for 3 years where fecal anti-CP23 IgA was able to be analyzed at the end of year one (n=376)) we were able to detect the previously observed delay in reinfection in High-IgA responders. We were able then to look at the amelioration of growth faltering (HAZ IgA high responders (Group 1): -1.323 ± 0.932 versus HAZ -1.731 ± 0.984 $p=0.0001$). High levels of anti-CP23 IgA rather than infection history was therefore associated with protection from one of the long-term health consequences of Cryptosporidium infections.

P8

Impaired host resistance to Salmonella during helminth co-infection is restored by anthelmintic treatment prior to bacterial challenge

Tara Brosschot (University of Victoria)

Intestinal helminth infection can impair host resistance to co-infection with enteric bacterial pathogens. However, it is not known whether helminth drug-clearance can restore host resistance to bacterial infection. Using a mouse helminth-Salmonella co-infection system, we show that anthelmintic treatment prior to challenge with Salmonella enterica serovar Typhimurium (S. Typhimurium) is sufficient to restore host resistance to Salmonella. To further elucidate the interkingdom relationship, we studied the spatial features of Salmonella

expansion during helminth infection. The presence of the small intestine-dwelling helminth Heligmosomoides polygyrus at the point of Salmonella infection supports the initial establishment of Salmonella specifically in the helminth's niche. We establish that when adult worms are present at the point of S. Typhimurium infection Salmonella remains largely in the lumen of the small intestine in close association with the adult worms, rather than invading host tissue. Interestingly, if helminth drug-clearance is delayed until Salmonella has already established in the small intestinal lumen, anthelmintic treatment reduces bacterial burdens but does not result in complete clearance of Salmonella. This suggests that while the presence of helminths supports initial Salmonella colonization, helminths are dispensable for Salmonella persistence in the host small intestine. In conclusion, we found that helminth drug clearance may be beneficial in reducing susceptibility to subsequent intestinal bacterial infections. However, helminth drug clearance after co-infection may not result in clearance of bacterial populations that have firmly established in the intestinal lumen. Our work suggests that the benefits of mass deworming in helminth-endemic areas may extend to attenuating gastrointestinal colonization by bacterial pathogens such as Salmonella.

P10

Inflammation at the interface: interplay between intestinal schistosomiasis and the host microbiota

Alice Costain (University of Manchester)

During mammalian infection with Schistosoma mansoni, parasite eggs transit from the mesenteric vessels across the intestinal wall and into the faeces. Despite this process being central to completion of the schistosome lifecycle, there are very few studies directly addressing the impact of egg transit at the intestinal interface on barrier integrity, microbial composition and intestinal immune responses. Here, we present a detailed characterisation of the intestinal environment during murine schistosomiasis, using a combination of high vs low dose, and mixed sex (egg production) vs male worm only (no egg production) infections. We show that intestinal permeability is significantly elevated during patent (egg producing) infections, and that infection intensity alters the kinetics of these barrier changes. Related to this, we show that patent infections are characterised by elevated serum levels of commensal-specific IgG, strongly suggesting increased bacterial translocation as a result of diminished barrier integrity. At an immunological level, patent infections induce type-2 dominated immune responses in the mesenteric lymph nodes and colonic lamina propria, as characterised by eosinophilia, an increase in Th2 cell cytokine production and transcription factor expression, and enhanced levels of type-2 associated factors (Relmα and YM1) within specific immune cell populations and in host faeces. Importantly, this dramatic type-2 shift coincides with significant alterations in the intestinal microbiota that become more marked over the course of infection and according to infection intensity. Finally, through the use of gnotobiotic mice and faecal transplants, we provide evidence that the schistosome-associated microbiota can significantly influence the character of the host immune response. In ongoing work, we are trying to determine which bacterial species (and/or their products) contribute to schistosome-mediated immunomodulation. Our data elevates mechanistic understanding of this interplay, providing new insight into parasite:host interactions that may aid in the design of future therapeutic strategies against schistosomiasis or other inflammatory diseases.

P12

Kupffer cells are not replaced by monocyte-derived cells and display both pro- and anti-inflammatory markers during Leishmania infantum infection

Gabriela Pessenda (NIAID/NIH)

Visceral leishmaniasis (VL) is a disease caused by Leishmania donovani/L. infantum and transmitted by sand fly bites. In the mouse model of VL, parasites establish infection in the liver and spleen. Although KCs have an embryonic origin, in some inflammatory settings they may be replaced by monocyte derived cells. In the L. donovani VL model, KCs have been shown to constitute the core of granulomas and to play an important role in the protective response. We aimed to further understand the role of KCs during L. infantum infection, focusing on the dynamics of granuloma formation, the extent of KCs replacement by monocyte-derived cells, and the activation programs of monocyte- and KC- populations during infection. By liver live imaging, KCs were the first cells to be infected by L. infantum. Clusters of KCs were readily detected, followed by neutrophils infiltration that interacted with infected KCs. At 19 days p.i., granulomas were identified, with eosinophils observed around a fused KC core. When KCs were depleted by clodronate-loaded liposomes, a drastic reduction in parasite burden was observed. However, when selectively depleted with diphtheria toxin, parasites were only slightly reduced. KC numbers were unaffected in CCR2-deficient infected mice, and monocytes transferred to infected mice did not differentiate into KCs. Single cell mRNA sequencing of CD45+ liver cells identified KCs in 4 different clusters, 3 of which also contained monocytes and monocyte-derived macrophages (mo-macs). By flow cytometry, Arg1 expression was downregulated and iNOS and PD-L1 were upregulated on KCs and mo-macs. On the other hand, TNF and Axl were upregulated mainly in KCs. Our data show that KCs rapidly initiate the process of granuloma formation after L. infantum infection and consist of a heterogeneous population expressing both pro- and anti-inflammatory markers, some also expressed by mo-macs. Although KCs are not replaced by monocyte-derived cells during infection, the tissue

environment appears to play an instructive role in establishing similar phenotypes on both populations of cells. Further investigation of how these heterogeneous populations are locally distributed through the liver and within granulomas will shed new light on how KCs and monocyte-derived cells contribute to parasite control.

P14

Understanding the mechanism of thrombocytopenia in malaria

Franklin Maloba (Mr)

A child dies of malaria every two minutes and most of these deaths are triggered by malaria-related complications such as anemia, respiratory distress and cerebral malaria. Thrombocytopenia occurs in most patients with Plasmodium falciparum and Plasmodium vivax during acute malaria disease and is an indicator of severe disease. The mechanism of thrombocytopenia in malaria is poorly understood and may arise from increased platelet consumption or suppressed platelet production from megakaryocytes in the bone marrow. Using the P. chabaudi non-lethal strain malaria we observed that platelet count reduces with increased peripheral parasitemia. Here we show that megakaryocytes and their progenitor cells decrease dramatically over the course of P. chabaudi infection and return to baseline after recovery from infection. We used transcriptomics analysis to determine that inflammation was present in the bone marrow, peaking around the same time of peak peripheral parasitemia with elevated levels of inflammatory cytokines such as interferon-ɣ (IFN-ɣ). IFN-ɣ has previously been shown to be responsible for contraction of myeloid progenitor cells in malaria. By comparing the transcriptomics of circulating platelets as a read out for megakaryocyte transcription, we saw that there was no change in the transcription of IFN-ɣ, but an increase in IFN-ɣR1 and IFN-ɣR2 indicating megakaryocytes respond to IFN-ɣ but are not the main instigators of this inflammatory molecule. There was an upregulation of the intrinsic apoptotic markers (Bax, Bak1, casp7 casp9) in the bone marrow and in platelets. Thus, our data support the hypothesis that thrombocytopenia in malaria may be driven by IFN-ɣ-induced apoptosis of megakaryocytes in the bone marrow, rather than platelet consumption.

P16

Definition of the pulmonary immune response in human and murine schistosomiasis

Emma L. Houlder (University of Manchester)

Infection with the parasitic worm Schistosoma mansoni causes considerable global morbidity, affecting over 200 million people, particularly in sub-Saharan Africa. Following skin penetration, the parasite migrates through the lung vasculature before maturation in the hepatic portal system, where it reaches patency and begins to produce eggs. Pulmonary immune responses to this parasite have so far been poorly defined. Here, we present detailed characterisation of schistosome-induced lung immune responses in lung migratory and patent infection of both humans and mice, utilising sputum as a proxy for lung responses in humans. We show that lung migratory and patent infection induces type-2 dominated immune responses in murine models, characterised by eosinophilia, an increase in Th2 cell cytokine production, and an increase in airway levels of soluble factors associated with type-2 responses (Relmα). In humans, trends for an increase in pro-inflammatory cytokines (IP-10) and chemokines (MCP-1) were observed during lung migratory infection. In contrast, during human patent infection no changes in pulmonary cytokines were observed, although systemic levels of the cytokine IP-10 significantly increased. Strikingly, expansion of conventional type-2 dendritic cells (cDC2s) was a conserved feature seen in both human and murine lungs at lung migratory and patent stages of schistosome infection. Mechanistically, we have shown that cDC2s are required to induce type-2 lung immune responses during murine lung migratory infection, and in future work we aim to assess if this is also the case in humans. This novel data elevates fundamental understanding of the pulmonary immune response during S. mansoni infection, information that may be relevant for future vaccine development, as well as providing mechanistic insight to help explain the impact of S. mansoni infection on allergic lung diseases such as asthma.

P18

Development of a spectral flow cytometry-based neutrophil-parasite killing assay

Julio Revilla (UVA)

Our group has shown that metabolic activity from the human gut commensal Clostridium scindens is linked to protection from infection with Entamoeba histolytica. Mice colonized with C. scindens exhibit elevated concentrations of serum deoxycholic acid (DCA) and bone marrow neutrophil progenitors. Following a challenge with E. histolytica, neutrophils in the gut increased significantly, suggesting a link between C. scindens colonization and the neutrophil response. Higher levels of serum DCA were attributed to C. scindens, as it can convert cholic acid into DCA. Removing C. scindens and supplementing mice with DCA instead achieved the same results. We aim to test the hypothesis that an increase in serum DCA alters the ability of neutrophils to kill amoeba. To measure amoebic killing we developed a

strategy for identifying and enumerating populations of live and dead amoeba using spectral flow cytometry. Amoeba were split into two groups, one incubated at 38°C, the other, heated at 100°C for 30 minutes as a control for dead amoeba. These samples of live and heat-killed amoeba were stained using a live/dead dye and a proliferation dye before being run on a spectral flow cytometer. Populations of live and heat-killed amoeba, along with their relative proportions, were identified in the software platform OMIQ using data gathered from the spectral cytometer. We intend to use this protocol to develop a model of neutrophil mediated amoeba killing utilizing cells derived from marrow from C. scindens colonized mice, DCA treated mice, and controls, to compare the phenotype and functionality of these neutrophils. Killing ability will be determined by the proportion of live and dead amoeba present in samples after being treated with neutrophils. This will further our understanding of the downstream effects DCA has on neutrophil activity during infection.

P20

Wild-derived mice as a model for asymptomatic malaria

Anne Jensen (University of Utah)

Individuals with asymptomatic malaria are a potential reservoir of disease transmission and pose a significant threat to the control of malaria worldwide. Asymptomatic malaria is usually defined as malaria without overt symptoms although mild anemia is often present and individuals tend to be more susceptible to bacterial co-infections. Asymptomatic malaria can occur at all ages. In older individuals asymptomatic malaria is thought to be associated with the development of the adaptive immune response after cumulative exposure to Plasmodium spp. However, in young children under the age of 2 asymptomatic malaria occurs prior to repeated exposures and the development of robust adaptive immunity. In this age group we hypothesize that differences in the immune response controlled by genetic variation of the host determines the outcome of disease. There is currently no immunologically intact mouse model for asymptomatic malaria. Here we utilize a specific pathogen free (SPF) wild-derived Mus musculus domesticus mouse strain with a similar amount of genetic variation to that of humans to demonstrate genetic control of asymptomatic malaria. When infected with Plasmodium yoelii XNL wild-derived mice display a large variation of anemia ranging from asymptomatic to severe. To explore the immunological correlates of anemia severity, we determine splenic responses at the peak of parasitemia (day 19 post-infection) and saw that innate immune response cytokines, in particular TNF-a and IL-10, were significantly correlated with the severity of anemia. This mirrors our findings in asymptomatic human populations from malaria endemic areas in Cameroon. In conclusion wild-derived mice can be used to model genetic diversity within human populations and this may enable us to pinpoint the immunological underpinnings of the asymptomatic malaria in children.

P22

Boromycin: rescuing an old yet a promising anti-apicomplexan drug

Jaypee Abenoja (Part- time)

Toxoplasma gondii and Cryptosporidium parvum, members of the phylum Apicomplexa, are significant pathogens of both humans and animals worldwide for which new and effective therapeutics are needed. Here we describe the activity of the antibiotic boromycin, previously described to inhibit gram positive bacteria and Eimeria sp., against Toxoplasma and Cryptosporidium. Boromycin potently inhibited intracellular proliferation of both T. gondii and C. parvum at half maximal effective concentrations (EC50) of 2.27 nM and 4.99 nM, respectively. Treatment of extracellular T. gondii tachyzoites with 25 nM of boromycin for 30 mins suppressed 84% of parasite growth, but T. gondii tachyzoite invasion into host cells was not affected by boromycin. Immunofluorescence of boromycin-treated T. gondii showed loss of morphologically intact parasites with randomly distributed surface antigens (SAG-1) inside the parasitophorous vacuoles. Boromycin exhibited a high selectivity for the parasites over their host cells. These results suggest boromycin is a promising new drug candidate for treating toxoplasmosis and cryptosporidiosis and could be an addition to anti-parasitic armamentarium.

P24

The Role of Phosphoenolpyruvate carboxykinase (PEPCK) in the Immunopathogenesis of Leishmania donovani infection.

Somtochukwu Stella Onwah (University of Manitoba)

Visceral leishmaniasis caused by Leishmania donovani is the most dangerous form of human leishmaniasis with an annual burden of about 400,000 new cases. Although there is no effective vaccine against human leishmaniasis, vaccination is possible because people who recover from natural infection are immune to reinfection. The lack of an effective vaccine against leishmaniasis is due to poor understanding of key immunodominant antigens and correlates of protective immunity in infected animals. We previously demonstrated that naturally processed peptide fragments PEPCK335–351 derived from Leishmania glycosomal Phosphoenolpyruvate carboxykinase (PEPCK) induced strong protective IFN-ɣ response following L. major infection. However, whether this enzyme plays an important role in visceral leishmaniasis, the

most medically important form of the disease is unknown. We hypothesize that deficiency of PEPCK in Leishmania donovani would result in a reduction in disease pathology and regulate host immune response. We generated PEPCK deficient (KO) and add-back (AB) strains of L. donovani using the CRISPR-Cas9 gene-editing technology. We confirmed the deletion and complementation of these genes in mutant and addback parasites, respectively by PCR and Western blot. We assessed this deficiency on parasite proliferation in axenic culture and bone marrow-derived macrophages in vitro. Growth kinetics in axenic culture and macrophages show that this deficiency results in reduced proliferation in comparison to wild-type (WT) and AB parasites. The attenuated phenotype of PEPCK deficient parasites in macrophages was not due to the disruption of metacyclogenesis which was confirmed by direct observation of morphological differences between procyclic and metacyclic promastigotes under an optical microscope. These findings indicate that PEPCK is an important metabolic enzyme of Leishmania and its deficiency results in impaired parasite proliferation in axenic culture and infected macrophages. Further studies in vivo, will assess the disease pathology in infected mice as well as their protective capacity upon rechallenge with WT parasites.

P26

Helminth infection activates tissue-resident T cell-mediated remodeling of the intestinal stem cell niche

Susan Westfall (Research Institute of McGill University Health Complex)

Intestinal helminths including Heligmosomoides polygyrus bakeri (Hpb) are coevolved pathosymbionts of mice that form a generally tolerated persistent infection. Although late-stage Hpb infection rears a type 2 immune response, we and others recently identified that the early invasive phase is dominated by a IFNg-driven type 1 immune response associated with intestinal stem cell (ISC) remodeling and disease tolerance to infection. Using a combination of cell depletion, adoptive transfer and cytokine-reporter approaches, we discovered that a subset of alpha-beta T cells was required for the type 1 immune response following Hpb infection. Within the T cell compartment, a population of IFNg+CD103+ tissue-resident memory CD8 T cells rapidly expanded at the site of infection in an antigen-independent manner. Importantly, depletion of T cells eliminated ISC remodeling. Collectively, our results suggest that damage-induced activation of tissue-resident memory T cells leads to reprogramming of the ISC niche to ensure epithelial barrier integrity and disease tolerance to infection.

P28

Chronic infection with Toxoplasma gondii activates monocytes leading to increased susceptibility to chemically-induced colitis

Iti Saraav (Postdoctoral Research Associate)

Prior studies have shown that acute Toxoplasma gondii infection in mice causes dysbiosis and ileitis both of which revert to normal over the course of chronic infection. However, it is unclear whether infection leaves a lasting impact on mucosal responses or not. Using a model for chemically induced colitis, we show that chronically infected mice display greater damage following treatment with DSS. Infected mice also showed impaired wound healing due to defect in stem cell regeneration of the epithelium. Enhanced susceptibility to DSS was not related to alterations in the microbiota, which returned to baseline in chronically infected mice, and which were similarly affected by DSS treatment in control and infected animals. Enhanced susceptibility was also not related to changes in Tregs that were unaltered in chronically infected mice. Rather, chronically infected mice showed persistently elevated levels of IFN-ɣ in CD4+ and CD8+ T cells and systemic activation of inflammatory monocytes that migrate to the intestine and produce inflammatory mediators. Enhanced tissue damage was attributable to inflammatory monocytes that emerge pre-activated from bone marrow and release inflammatory mediators including nitric oxide once they reach inflamed tissue sites. Blocking monocyte recruitment or use of Nos2-/- mice that lack inducible nitric oxide synthase, protected chronically infected animals from DSS associated intestinal damage. Although heightened innate immunity contributes to protection, our findings demonstrate that such responses not always beneficial as pre-activated monocytes also increased tissue damage in response to DSS. Together, our work uncovers a mechanism for persistent monocyte activation during chronic T. gondii infection that may increase the risk of inflammatory diseases.

P30

Sm16, a Schistosoma cathelicidin-like immuno-modulator

Richard Lalor (National University of Ireland Galway)

Sm16 is a low molecular weight protein (~16kDa) secreted by Schistosoma mansoni, a causative agent of human schistosomiasis. The molecule is expressed in the cercariae and eggs stage of S. mansoni but not in adult worms suggesting that it plays an important role in the invasive stages through the skin but also in egg migration out of the host. Phylogenetic, structural and functional analysis of Sm16 provides evidence for its inclusion within the helminth defence molecule (HDM) family, potent immune-modulators exclusive to trematode species (first described in Fasciola hepatica). Like HDMs, Sm16 contains an amphipathic helix which spans the C-terminus. We have

shown that a synthetic peptide derivative of the C-terminal section (residues 31–115, Sm16-KS84) performs an immune-modulatory function. Sm16-KS84 alone was shown to bind and enter immune cells (macrophages), eliciting a weak pro-inflammatory response, which may partially account for the pro-inflammatory response to eggs observed in liver tissues. However, it also blocked the pro-inflammatory effects of bacterial endotoxin when co-incubated with macrophages. The C-terminal amphipathic helix was shown to be critical for this immunosuppressive activity. A shortened synthetic peptide encompassing this helical structure (Sm16-KS66) was shown to exhibit greater suppressive effects, but also no longer induced a pro-inflammatory response. Similar to HDMs, this fragment inhibited NLRP3 inflammasome activation, via inhibition of vATPase induced lysosomal acidification. However, unlike other HDMs, the mechanism by which it suppresses bacterial endotoxin was mediated by the extracellular neutralisation of the antigen. This was attributed to a sequence within Sm16 that shares high structural and functional homology with human and bovine cathelicidins (LL-37 and BMAP respectively), host defence peptides with antimicrobial and immunomodulatory functions. This would suggest that Sm16 has diverged from HDMs as more cathelicidin-like immune-modulators. We suggest that these small synthetic form peptides have immuno-therapeutic potential as they exhibit potent bioactive and immunomodulatory properties.

P32

Modulation of host macrophage mitochondrial metabolism by Leishmania donovani requires the surface coat glycolipid lipophosphoglycan

Hamlet Acevedo Ospina (INRS - Centre Armand-Frappier Santé Biotechnologie, Canada)

To colonize macrophages, Leishmania metacyclic promastigotes employ virulence factors, including lipophosphoglycan (LPG), which impair different host cell processes. Whereas previous studies revealed that Leishmania alters signaling axes that regulate mitochondrial function, scarce attention has been paid to the characterization of host cell mitochondrial metabolism during Leishmania infection and to the effectors involved therein. In this study, we addressed the hypothesis that L. donovani modulates host cell mitochondrial metabolism and function in an LPG-dependent manner. We obtained evidence that L. donovani promotes host cell mitochondrial biogenesis in an LPGdependent manner. Hence, we observed an increase in the mitochondrial/nuclear DNA ratio which corresponded to an upregulation of host cell nuclear and mitochondrial genes involved in mitochondrial biogenesis. Western Blotting analyses revealed an overexpression of genes involved in the ETC complex, along with an increase in AMPK phosphorylation on Thr172. Infection with L. donovani also resulted in an LPG-dependent increase in mitochondrial fluxes, as indicated by the higher ratio of oxygen consumption over extracellular acidification. Importantly, this process was dependent on glycolysis, but independent of β-oxidation, glutaminolysis or peroxisomal β-oxidation. Using macrophages from knockout mice, we demonstrated that L. donovani promastigotes promote a rapid up-regulation of IFN-α via TLR4 and endosomal TLRs. Similarly, the induction of host mitochondrial biogenesis required TLR4, endosomal TLRs, and IFNAR- 1. These results reveal the role of different pattern-recognition receptors in the induction of IFN-α, thus suggesting that IFN-α regulates the mitochondrial biogenesis process in an inflammatory context. Finally, pre-treatment of BMM with AICAR, which stimulates mitochondrial biogenesis, promoted the replication of L. donovani, highlighting the importance of mitochondrial biogenesis in the colonization process. Collectively, our data provide novel insights into the mechanisms by which Leishmania metacyclic promastigotes alter host cell mitochondrial dynamics during the colonization process.

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P34 – Duplicate

P36 – Withdrawn

P38

In vitro anti-inflammatory effect of Echinococcus granulosus laminated layer during human Rheumatoid Arthritis

Fahima Ameur (Team “Cytokines and NOSynthases”, Laboratory of Cellular and Molecular Biology (BCM), Faculty of Biological Sciences (FSB), University of Sciences and Technology Houari Boumediene (USTHB))

Background: The “Hygiene Hypothesis” proposes that helminthes infections, especially during childhood, can protect against allergic and other inflammatory autoimmune diseases. Chronic helminthes infections are characterized by the ability to induce regulatory responses in the host that can help the control of inflammation. Many studies have focused on the immunomodulatory effect of the laminated layer (LL) of the hydatid cyst of Echinococcus granulosus. The main goal of this work is to investigate the in vitro immunomodulatory effects of the LL in Algerian Rheumatoid arthritis (RA) patients.

Methods: Mononuclear cells (PBMCs) of active and inactive RA patients and healthy controls (HC) were collected and stimulated with different concentrations of LL extract for 24 h. Moreover, cells were also treated with anti-TNF-α, anti-IL-6, IL-17, PHA (T lympohocytes inducer), L-arginine and L-ornithine (respectively NOS substrate, NOS inhibitor at high concentration) without or with LL. We then evaluated the nitric oxide (NO) levels and extracellular matrix metalloproteinases (MMPs) activities in cultures supernatant, respectively by the Griess method and gelatin zymography. Finaly, the expression of iNOS and NF-κB were analyzed in PBMCs by immunofluorescence test. Results: Our study revealed higher NO production and MMP 9/2 activities in PBMC culture of active RA patients compared to inactive patients and HC. Interestingly, the treatment of PBMC with LL, anti-TNF-α, anti-IL-6 and L-ornithine inhibits NO production and MMPs activities in RA patients. These effects are associated with a diminution of iNOS and NF-κB expression.

Conclusion; Our results demonstrate the immunomodulatory and anti-inflammatory effects of hydatid laminated layer in RA. The potential therapeutic effect of LL in RA is under investigation in a mouse model of RA.

P40 – Duplicate

P42

Effect of prophylactic vaccination with the membrane-bound acid phosphatase gene of Leishmania mexicana in the murine model of cutaneous leishmaniasis

Maria A. Burgos Reyes (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional)

Leishmaniasis is a disease caused by an intracellular protozoan parasite of the genus Leishmania. Current treatments for leishmaniasis are long, toxic, expensive, and are not available in some endemic regions. To date, there is not an effective vaccine for the prevention and elimination of human leishmaniasis. In this study, the LmxMBA gene of Leishmania mexicana was selected as a possible vaccine candidate using the reverse vaccinology approach. The prophylactic effect of DNA vaccination with this gene in a murine model of cutaneous leishmaniasis was evaluated. BALB/c mice were immunized with the plasmid pVAX1::LmxMBA and subsequently infected in the footpad with promastigotes of L. mexicana. Plasmid pVAX1 (empty vector) and phosphate-buffered saline (PBS) were used as control groups. Infected tissue sections were obtained for histological analysis and to determine the parasitic load. Additionally, the serum and spleen were removed from the mice to analyze the humoral and cellular immune responses. The results showed that prophylactic vaccination with pVAX1::LmxMBA significantly reduced the lesion's size and the parasitic load on the footpad compared to the control groups. At a histological level, a smaller number of parasites was evident in the dermis and the absence of connective tissue damage. Mice immunized with plasmid pVAX1::LmxMBA induced immunity characterized by an increase in the IgG2a/IgG1>1 ratio and a higher rate of lymphocyte proliferation. In this study, immunization with the plasmid promoted an improvement in the macroscopic and microscopic clinical manifestations of the experimental infection by L. mexicana, with a T helper 1 response characterized by an IgG2a/IgG1 >1 ratio and high antigen-specific lymphoproliferative response. These findings support immunization with the plasmid pVAX1::LmxMBA as a preventive strategy against cutaneous infection of L. mexicana.

P44

Low molecular weight protein tyrosine phosphatase (LMW-PTP2) protein can potentially modulate virulence of the parasite Entamoeba histolytica.

Diana Milena Torres-Cifuentes (Cinvestav Mexico)

The Entamoeba histolytica parasite is the causative agent of amoebiasis, infecting approximately 1% of the world's population and causing 100,000 deaths per year. Trophozoites adhere to Fibronectin (FN), activating signaling pathways regulated by kinases and phosphatases. The genome of E. histolytica encodes for low molecular weight tyrosine phosphatase proteins (EhLMW-PTPs) that are expressed in trophozoites and amoebic cysts. In other organisms, these proteins regulate cell proliferation, motility, and adhesion, and in humans, they have been classified as potential oncogenic genes. However, in the parasite, the role of these phosphatases has not yet been well characterized. Our results showed that the EhLMW-PTPs proteins' genes have a differential expression in trophozoites of the HM1:IMSS strain during asynchronous cultures. Likewise, we found that the substrates of 6HisrEhLMW-PTP2 were low molecular weight proteins involved in the actin cytoskeleton structuration. Furthermore, we observed that the transfected trophozoites that overexpressed the EhLMW-PTP2 protein phagocytized fewer erythrocytes, possibly due to decreased phagocytic cup formation, and also showed deficiencies in adhesion to FN, less cytopathic effect. Trophozoites transfected with the EhLMW-PTP2 mutant showed a decreased liver abscesses development in the hamster model. These results suggest that the parasite's EhLMW-PTP2 can potentially modulate the virulence of E. histolytica as it participates in adhesion, migration, and phagocytosis.

P46

IL-27 impacts emergency monopoiesis and monocyte function during acute Toxoplasma gondii infection

Daniel Aldridge (University of Pennsylvania)

IL-27 is a pleotropic cytokine composed of the p28 and EBi3 subunits. During infection with the protozoan parasite Toxoplasma gondii, mice that lack IL-27 succumb to severe, infection-driven immunopathology. While this pathology is driven by aberrant CD4+ T cell responses at 7-10 days post-infection (dpi), we have observed that as early as 5dpi the absence of IL-27 results in marked changes in the innate immune cell compartment, characterized by an enhancement in an inflammatory monocyte gene signature. Intriguingly, murine monocytes do not express the IL-27 receptor, suggesting that monocytes must be affected indirectly by the absence of IL-27 during infection. Hematopoietic stem cells and other monocyte progenitor cells do express the IL-27 receptor, and IL-27 has been shown play a role in controlling the differentiation of these cells during emergency granulo- and myelopoiesis. To determine, then, whether the loss of IL-27 during toxoplasmosis results in abnormal differentiation of monocyte precursor cells, we examined this during the infection of mice lacking either the p28 or EBi3 subunits of IL-27 (p28-/- and EBi3-/-). In these experiments p28-/- mice had an enhancement in the precursor populations associated with monopoiesis, and we saw a similar enhancement in mice lacking the EBi3 subunit of IL-27. Additionally, EBi3-/- mice showed an increase in the numbers of TNFγ producing monocytes. Taken together, these data suggest a novel function of IL-27 in restraining monopoiesis and the generation of inflammatory monocytes.

P48

In vitro immunoregulatory activity and anti-inflammatory effect of Echinococcus granulosus laminated layer

Sara Benazzouz (Team “Cytokines and NOsynthase, Immunity and pathogeny” Laboratory of cellular and molecular biology, faculty of biological sciences, University of science and technology Houari Boumediene)

The Laminated layer (LL) of Echinococcus granulosus (EG) plays a very important role in protecting the metacestode from host immunity. In this current study, we investigated the immunomodulatory effect of the LL on mouse spleen cells in presence of LPS and its characterization. Mouse spleen cells were co-cultured with/without LL in presence of LPS. After 24 hrs, NO was measured in culture supernatant by Griess-modified method. The mRNA expression levels of cytokines (IFN-gamma, IL-1beta TGF-beta, IL-10), Foxp3 , CTLA-4 were measured by qRT-PCR. Frequency of Foxp3, IL-10 and IFN-gamma in CD4+ T cells were measured by using flow cytometry (FACS). SDS-PAGE, Western Blot (WB) and amino-acid analysis (AAA) were used for LL protein characterization. Our results showed a significant decrease ($p< 0.01$) in NO production and IFN-gamma mRNA level ($p< 0.001$) from mouse spleen cells in response to LPS. LPS-induced high level of IL-1beta that was significantly ($p<0.05$) down regulated by LL. Interestingly, mRNA levels for TGF-gamma ($p< 0.01$), Foxp3 and IL-10 ($p< 0.05$) were significantly upregulated by LL. FACS results showed increased frequencies of CD4+ Foxp3+ and LPS-induced high CD4+ IFN-gamma+ frequency that were decreased in LPS+LL treated cells. Our data showed the immuno-regulatory and anti-inflammatory effects of the LL likely through Treg cells and related cytokines (IL-10/TGF-beta), and opens perspectives to study the therapeutic or preventive potential of LL during inflammatory diseases.

P50

cDC1 are Essential for the Survival but not Priming of CD8+ T cell Responses during T. gondii Vaccination

David Christian (University of Pennsylvania)

Type 1 conventional dendritic cells (cDC1) have been shown to be critical during T. gondii infection as an early source of interleukin 12 (IL-12) as well as for the induction of the CD8+ T cell response. Similarly, after intraperitoneal immunization with a replication deficient strain of T. gondii, IL-12 from cDC1 induced innate lymphoid cells to produce interferon gamma required to recruit inflammatory monocytes to the site of immunization. Batf3-/- mice that lack cDC1 demonstrated an inability to induce a protective, parasite-specific CD8+ T cell response. Surprisingly, this CD8+ T cell defect was not a result of an inability to prime CD8+ T cells as infected macrophages in the peritoneum are shown to migrate to the omentum where they can present parasite antigen to CD8+ T cells via MHC class I. Instead, the absence of cDC1 results in parasite-specific CD8+ T cells that are defective in upregulating nutrient and cytokine receptors and exhibit increased rates of cell death in early divisions after priming. This loss of T cells in early divisions results in the significant decrease of CD8+ T cells at later time points that subsequently fail to protect upon lethal rechallenge.

P52

Impact of ether lipids on virulence and host immune response to Leishmania major

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Introduction: Leishmaniasis is a spectral disease with clinical manifestations ranging from mild self-healing skin ulcers to chronic muco-cutaneous infection and severe systemic infection. While drugs are available for treating the disease, most are expensive or highly toxic. Interestingly, recovery from Leishmania infections leads to long-lasting protective immunity, suggesting that the disease can be prevented through vaccination. A key challenge is determining the antigens that could either be used as recombinant vaccine candidates or targeted for the generation of attenuated parasite to be used as live-attenuated vaccine. Alkyl-dihydroxyacetone phosphate synthase (ADS) is the critical enzyme involved in the biosynthesis of glycerol-containing ether lipids, which is required for the synthesis of glycosylphosphatidylinositol (GPI). GPI is important for anchoring lipophosphoglycan (LPG) and gp63, which are major virulence factors of the parasite, to the cell membrane. Deficiency of ADS synthesis leads to impaired synthesis of GPI-anchored molecules resulting in attenuated virulence. However, the impact of ADS deficiency on the immunopathogenesis of cutaneous leishmaniasis has not been studied.

Methods: The growth kinetics of ADS deficient (ADS KO) L. major parasite in axenic culture were compared to wild-type (WT) and Add-back (ADS-AB) parasites in axenic culture. Also, bone marrow-derived macrophages were infected with WT, ADS KO and Add-back parasites and infectivity and parasite proliferation were measured and compared at different times after infection by staining cytospin preparations with Giemsa stain and counting under a light microscope.

Results: Our preliminary study shows that ADS gene deficiency affects the growth kinetics of L. major in axenic culture. In addition, ADS deficient parasites showed lower macrophage infectivity in vitro compared to their wild-type (WT) controls.

Conclusion: Deficiency of ADS in Leishmania major affects parasite growth rate in axenic culture and infectivity in macrophages in-vitro, confirming the critical role of GPI-anchored in parasite proliferation and infectivity. Further studies will assess the impact of ADS gene deficiency in disease pathogenesis and host immune response in infected mice.

P54

Staphylococcus aureus contributes to cutaneous leishmaniasis pathology by stimulating neutrophil skin infiltration and enhancing pro-inflammatory gene expression

Camila Farias Amorim (University of Pennsylvania)

Patients infected with Leishmania braziliensis exhibit changes in the skin microbiome, most often associated with a predominant colonization by Staphylococcus. To understand how a dominant Staphylococcus-like microbiome influences the local immune responses in cutaneous leishmaniasis, we collected lesion biopsies, bacterial isolates, and lesion swabs for 16S sequencing from 64 patients infected with L. braziliensis in the Northeast of Brazil. These patients had localized lesions and the samples were collected prior to treatment. We found that the patients exhibited a dysbiotic skin microbiome at the lesion site when compared with the contralateral skin which in most patients was dominated by Staphylococcus. Since S. aureus was the most isolated bacteria from those Staphylococcus dysbiotic lesions, we investigated its influence on host gene expression. We quantified S. aureus in the lesions by qPCR and by aligning RNA-seq reads to the bacterial reference genome. We observed a significant association between high S. aureus burden and increased neutrophil abundance (xCell RNA-seq workflow), accompanied by an enrichment of genes associated with cell recruitment (such as CXCL8). Moreover, the S. aureus burden was associated with enhanced expression of genes encoding for components of the extracellular matrix (metalloproteinases, collagen, and laminin), for pro-inflammatory cytokines (IL1A, IL1B, IL36A), and defense to bacteria (GNLY and defensin genes). In our experimental murine models of cutaneous leishmaniasis these immune responses are associated with increased disease rather than parasite control, which suggests that S. aureus may contribute to the immunopathology associated with L. braziliensis infections.

P56

Diverse pulmonary macrophage populations contribute to host-protection following a helminth challenge

John Ponessa (Rutgers New Jersey Medical School)

Helminth infections are a major cause of morbidity in the developing world and represent a significant public health concern. 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 by mediating reductions in parasitic burdens and promoting 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). Although these populations are easily identified and appear phenotypically and functionally distinct initially, recent research has now revealed that Mo-Macs acquire 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 'fate mapper' mice, which allow us to observe changes that occur as Mo-Macs supplement TD-Macs 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 also acquire the expression of Carbonic anhydrase 4 (Car4), a lineage identifying gene of TD-Macs, after entering the lung microenvironment. To further interrogate the role Car4 plays in programming of tissue-resident macrophages, we generated novel Car4-floxed mice to perform linage-specific deletion experiments. Critically, genetic deletion of Car4 in CD11c+ cells resulted in reduced pulmonary function and diminished alveolar macrophage populations at baseline and post-Nb infection. Together, these data suggest Car4 may regulate alveolar macrophage function and development.

P58

Age-dependent Humoral Profiles Identify Asymptomatic Malaria Infection with Minimal Set of Markers

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As we come closer to elimination of malaria in endemic areas of the world, there is a greater need for a field-deployable diagnostic for asymptomatic malaria. Traditional antibody-based serologic diagnostics based on immunodominant Plasmodium falciparum antigens have been impossible due to the high prevalence of malaria-specific antibodies in endemic regions. To identify antibody profiles that might distinguish individuals with asymptomatic P. falciparum infection from uninfected individuals in Mali, we performed systems serology profiling of antibody titer, subclass, and Fc-directed functions of 80 P. falciparum proteins. Using machine learning algorithms, we identified a four-antigen panel that had 88% classification accuracy. Further, we found that antibody profiles of asymptomatic infection changed as children aged. Altogether, our results suggest the potential of using antibody functional profiles to develop a serologic assay for asymptomatic malaria.

P60

Group 1 Metabotropic Glutamate Receptor's Influence on T cells in T. gondii Infection

Edward A. 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 parasite reactivation. Infection in the immunocompromised leads to lethal Toxoplasmic encephalitis while in the immunocompetent, there is persistent low-grade inflammation which lacks clinical symptoms. This suggests that there is a tightly regulated inflammatory response to T. gondii in the brain. T cells are the dominant immune cell that control recrudescence and parasite replication through secretion of effector molecules such as perforin and IFN-ɣ. However, the regulation of these cells in this tissue is poorly understood. During chronic infection there is an increase in extracellular (EC) glutamate. High extracellular glutamate is not specific to T. gondii infection and can occur during multiple pathologies 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 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, expression can be determined by T cell phenotype. We further hypothesize that T cells recruited to the brain are regulated by glutamate through group 1 metabotropic glutamate receptor modulation. Using activators and inhibitors of these receptors 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. What we learn here could be applied to models of neurodegenerative disease, as some of these neurological disorders similarly modulate glutamate signaling and recruitment of T cells to the CNS. Understanding the effect of exogenous glutamate on immune cells in the brain is a critical avenue to explore as changes in glutamate concentrations may disrupt the delicately balanced inflammatory response to T. gondii.

P62

Unraveling immunostimulatory proteins in Haemonchus contortus soluble antigens

Si Wang (China Agricultural University)

The multicellular parasites (helminths) have evolved with their hosts for millions of years. To establish a long-lasting infection, helminths secret various immunomodulating molecules into their hosts. The blood-feeding gastrointestinal nematode, Haemonchus contortus, is one of the most pathogenic parasites in small ruminants. Consistent with other helminth infections, H. contortus polarizes host

immune responses toward an anti-inflammatory/ Th2 phenotype. However, little is known about the immunostimulating molecules in H. contortus that induce the anti-inflammatory/Th2 skewing. Here, we performed an unbiased screen to identify immunostimulant in H. contortus that may induce IL-4 expression in sheep PBMCs. Haemonchus contortus soluble antigens (HcAg) were separated into multiple components by size-exclusion chromatography (SEC) and ion-exchange chromatography (IEC), followed by mass spectrometry identification. Recombinant expression of several candidate proteins in eukaryotic cells verified their IL-4-inducing property. Notably, most of these proteins were previously identified as excretory/secretory antigens during the blood-feeding stages of H. contortus, suggesting their potential roles in Th2 skewing.

P64

Leishmania braziliensis exossomes trigger inflammatory response in human macrophages

Fabio Peixoto (LAPEC - Fiocruz, BA)

Background: Exosomes are 30-100nm extracellular vesicles secreted by a variety of eukaryotic cell. Leishmania exosomes are composed by proteins such as heat shock proteins, annexins, GP63 and proteins with signaling activity, as well as mRNAs and miRNA. It has been reported that Leishmania donovani exosomes activate tirosine-phosphatases, downregulating IFN-ɣ and inhibiting the expression of microbicidal molecules such as TNF and NO, thus contributing to parasite replication. Studies have suggested that after a first stimuli mononuclear phagocytes passes through epigenetic modifications that increases its effector mechanisms against a second stimuli. L. braziliensis infection induces macrophages to produce high levels of TNF and IL-1β known to be involved in skin ulcer development in cutaneous leishmaniasis patients. Our hypothesis is that the sensitization of macrophages with L. braziliensis exosomes prior to the infection by this pathogen, increase inflammatory cytokines production, as well as inflammasome activation. Methods and Results: Healthy subjects macrophages were sensitized with L. braziliensis exosomes and then infected with L. braziliensis. Cytokines concentration and parasite counts were determined. Our results show that purified exosomes induce IL-1β and TNF in macrophages. We also observed higher levels of IL-1β in cultures that were sensitized with exosomes prior to infection, when compared to L. braziliensis-infected cells without been stimulated with exosomes. Exposure of macrophages to L. braziliensis exosomes prior to in-vitro infection did not change the percentage of infected macrophages or number of amastigotes within these cells overtime. Our results show that exosomes from L. braziliensis induce inflammatory responses without affect parasite killing, suggesting the participation of these vesicles in the pathogenesis of the disease.

P66

The role of mitochondrial and lysosomal permeabilization in eastern oyster apoptotic response to Perkinsus marinus

Erin M. Roberts (University of Rhode Island)

Apoptosis, or programmed cell death, is part of a complex, innate-immune response to Perkinsus marinus infection in the Eastern oyster, and apoptosis of infected hemocytes may reduce parasite replication. Previous studies revealed apoptosis stimulation shortly following P. marinus infection, but the parasite may be able to limit apoptosis as infection proceeds. Specific organelles and pathways mediating the apoptotic response to P. marinus are unknown. Eastern oysters were challenged in vivo with P. marinus, and hemocyte apoptosis, caspase 3/7 activation, and lysosomal permeabilization were investigated 7 d post-infection using flow cytometry. Granular hemocyte apoptosis significantly decreased in challenged oysters as compared to control oysters, indicating possible inhibition by the parasite. Caspase 3/7 activation and lysosomal permeabilization were not significantly affected by P. marinus, indicating likely involvement of a caspase-independent pathway in hemocyte response and no involvement of lysosomal permeabilization. Oyster hemolymph samples were challenged in vitro with P. marinus at four multiplicities of infection (MOIs; P. marinus to hemocyte 1:1, 5:1, 10:1, and 25:1) for 1 hr, and hemocyte apoptosis and mitochondrial permeabilization were investigated with flow cytometry. Granular hemocyte apoptosis increased at all MOIs compared to control, although levels of mitochondrial permeabilization did not change significantly, suggesting mitochondria are not involved in the apoptotic response to P. marinus. Further research explores the role of Inhibitor of Apoptosis Proteins (IAPs) in modulating hemocyte apoptosis in response to P. marinus challenge. Uncovering pathways regulating Eastern oyster apoptotic response to Dermo disease may aid in targeting genes for breeding for disease resistance.

P68

Brain-resident neutrophils contribute to protection against chronic 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. Despite being mostly asymptomatic in immunocompetent hosts, infection stimulates the robust recruitment of both innate and adaptive immune cells to maintain balanced control over the parasite. While adaptive T cells have long been identified as one of the main immune modulators of chronic central nervous system (CNS) infection, there is a new appreciation of innate immune cells acting beyond acute periods of infection. Previously, other groups have shown neutrophils entering the brain early during Toxoplasma infection. Now, using flow cytometry, we demonstrate that neutrophils remain present in the brain through the course of chronic infection. Depletion of these neutrophils suggests this population is required for control of the parasite. Further characterization of this chronic population reveals novel subpopulations and expression of the neuroprotective and regulatory molecules NRG1, ErbB4, and MSR1. Additional experiments address the location and role of these neutrophils in the CNS. Collectively, these results demonstrate a novel chronic brain neutrophil population that may play an alternative role in controlling chronic infection and inflammation in the CNS.

P70

CD8+ T cells provide protection during Cryptosporidium Infection

Breanne Haskins (University of Pennsylvania)

Cryptosporidium is an enteric pathogen that resides in an unusual intracellular, yet extracytoplasmic, subcellular location in intestinal epithelial cells. Due to the unique niche Cryptosporidium occupies, it is unknown how antigen is acquired by the immune system to activate a protective immune response, and it is unclear how T cells mediate resistance. For example, while T cell production of IFNg is required for parasite control, there is also evidence of an IFNg-independent, T cell-dependent mechanism of resistance. To study the T cell response to Cryptosporidium, we developed a novel model in which Cryptosporidium has been engineered to express SIINFEKL peptide. This allows us to use transgenic OT-I CD8+ T cells specific for SIINFEKL as a surrogate of Cryptosporidium-specific CD8+ T cells. In this model, I have already shown that these parasites induce a potent CD8+ T cell response, and that transfer of OT-I cells can promote control of Cryptosporidium. To determine how CD8+ T cells mediate protection, I infected IFNg-Thy1.1 reporter mice with SIINFEKL-expressing parasites. Indeed, CD8+ T cells produce IFNg during infection, and some were also specific to SIINFEKL antigen. This suggests that CD8+ T cell production of IFNg is protective during infection. However, it is unknown how CD8+ T cells are primed to provide this protection. To determine if cDC1s, the dendritic cell (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. These data suggest that cDC1s are critical for robust CD8+ T cell responses during Cryptosporidium infection. Together, my findings indicate that CD8+ T cells are protective during Cryptosporidium and this response requires DCs.