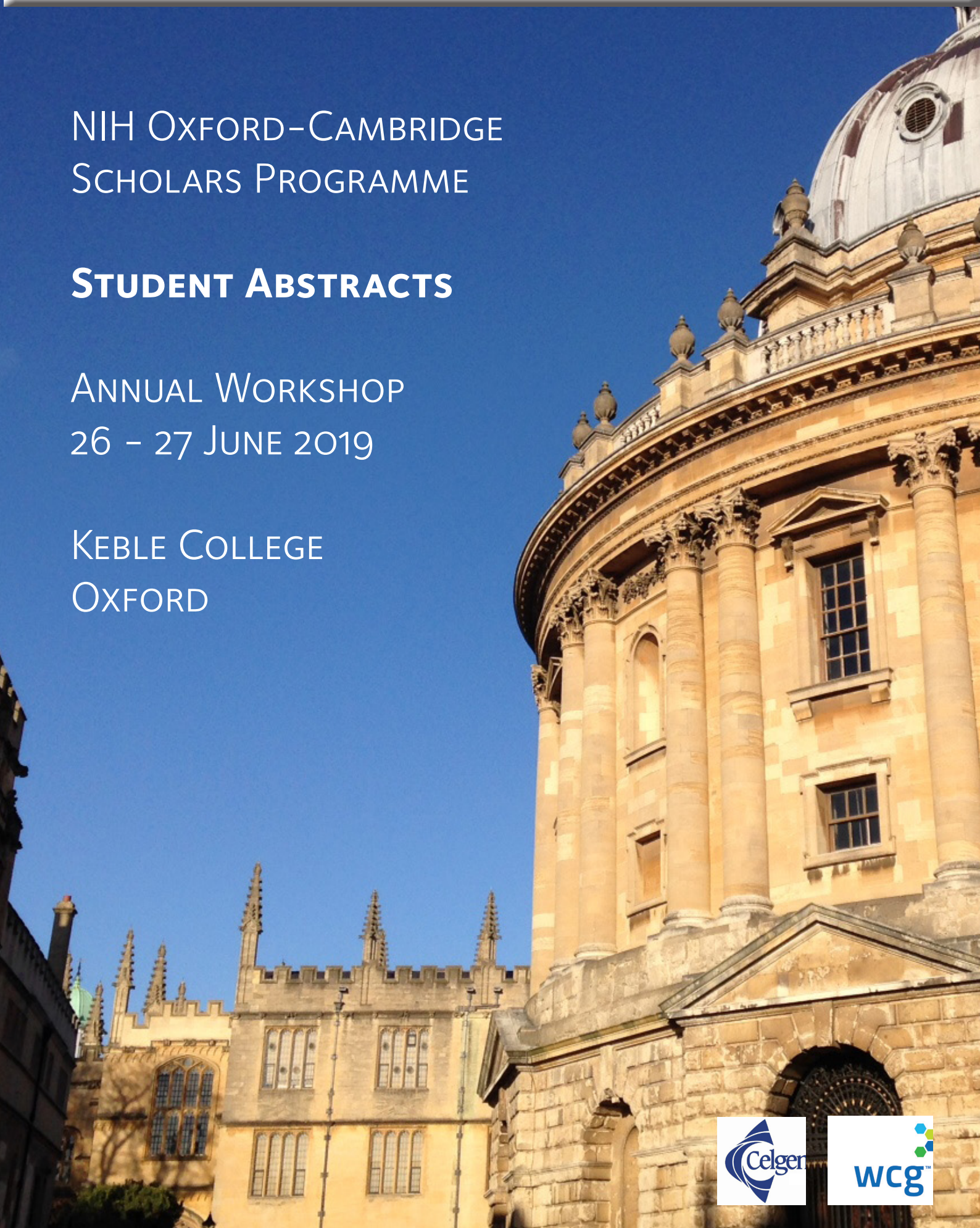

NIH Oxford–Cambridge Scholars Programme

Student Abstracts

Annual Workshop
26 – 27 June 2019

Keble College
Oxford

Student Presentation Abstracts

Wednesday 26 June: 11:30 - 12:45
Thursday 27 June: 09:30 - 10:45 and 14:00 - 15:00

Nicholas Ader
Erin Coonahan
Michael Fernandopulle
Zachary Fitzpatrick
Kristoffer Haurum Johansen
Samuel Katz
Hannah Mason
Joseph McAbee
Shannon Jane McKie
Nicole Mihelson
William Nathan
Gianmarco Raddi
Joseph Roney
Zinan Zhang

Student Poster Abstracts

Wednesday 26 June: 16:30
Thursday 27 June: 16:00

Victoria Avanzato
Benjamin Badger
Kathleen Bashant
Adriano Bellotti
Daniel Bronder
Charles Coomer
Madeline Epping
Taylor Farley
Cameron Gardner
George Heaton
Stewart Humble
Aleksandra Ivovic
Mindaugas Jonikas
Emily Kolyvas
Bridget Larman
Jacob Levenstein
Jonathan Liang
Connie Mackenzie-Scott
Christian Márton
Allison Meadows
Michael Metrick
Matthew Mulè
Laura Palmieri
Ryan Prestil
Jyothi Purushotham
Lindsey Rosen
John Shannon
Samantha Tilson
Jessica van Loben Sels
Alex Waldman
Lawrence Wang
Lauren Wedekind
Margaret Westwater-Wozniak
Audrey Winkelsas
Derek Xu
Zinan Zhang
Yifan Zhou

Molecular and topological reorganization in mitochondrial architecture interplay during Bax-mediated steps of apoptosis

Abstract Authors	Nicholas R. Ader, Patrick C. Hoffmann, Iva Ganeva, Alicia C. Borgeaud, Chunxin Wang, Richard J. Youle, and Wanda Kukulski
Graduate Student Name	Nicholas R. Ader
NIH Institute-Center	NINDS
NIH Research Mentor	Richard Youle
UK Graduate University	Cambridge
University Research Supervisor	Wanda Kukulski
Graduate Department/Program	MRC Laboratory of Molecular Biology

During apoptosis, Bcl-2 proteins such as Bax and Bak mediate the release of pro-apoptotic proteins from the mitochondria by permeabilizing the outer mitochondrial membrane. Bax/Bak are known to insert into the outer membrane, oligomerize into clusters and thereby generate ruptures of several hundred nanometers in the outer membrane. However, it remains unclear how such large outer membrane openings form. Furthermore, inner membrane remodeling is also required for release of pro-apoptotic factors, but how that is coupled to outer membrane rupturing remains elusive.

Here, we combined different correlative microscopy and electron tomography approaches to visualize the effects of Bax activity on mitochondria in human cells. In particular, we employed electron cryo-tomography on cryo-focused ion beam-milled cells to visualize the native cellular ultrastructure at high resolution.

Our data show that Bax clusters localize near outer membrane ruptures of highly variable size. While these clusters appeared amorphous in electron tomograms of resin-embedded cells, electron cryo-tomography of vitreous cells revealed structural elements indicating a higher-order organization of their components. This structure was reminiscent of a sponge-like meshwork and suggested that Bax-associated outer membrane components may get sequestered into clusters, thereby contributing to rupture formation. We also observed that ruptured mitochondria displayed diverse inner membrane rearrangements, including unfolding of inner membrane cristae and compartment dilution. We noted that unfolding of cristae is coupled to changes in dimerization and distribution of ATP synthases, particularly pronounced at membrane segments exposed to the cytosol by ruptures. Furthermore, the degree of cristae unfolding correlated with the size of outer membrane rupture, suggesting that inner membrane flattening contributes to rupture formation.

Based on our results, we propose a comprehensive model in which molecular reorganizations of the inner membrane and sequestration of outer membrane components into Bax clusters interplay in the formation of outer membrane ruptures.

An aptamer-based assay for the detection of antimalarial drugs

Abstract Authors	Erin Coonahan, Kyung-Ae Yang, Maarten De Vos, Joel Tarning, Thomas E. Wellems, Carole A. Long
Graduate Student Name	Erin Coonahan
NIH Institute-Center	NIAID
NIH Research Mentor	Carole Long
UK Graduate University	Oxford
University Research Supervisor	Maarten De Vos, Joel Tarning
Graduate Department/Program	NDM

Artemisinin-based combination therapies (ACTs) have begun to fail as first-line therapies for the treatment of Plasmodium falciparum malaria in Southeast Asia. Preventing the further spread of drug-resistant parasites is a top priority for global malaria elimination campaigns. A low-cost, field-based assay to detect slow-clearing ACT compounds from patient samples would allow for the tracking of antimalarial drug use, monitoring of drug compliance, and assessments of therapeutic efficacy and resistance. It could also provide sensitive detection of active components in assessments of tablet quality.

We have developed a low-cost, rapid, fluorescent sensor for the specific detection of piperaquine and mefloquine, two of the most commonly used drugs in Southeast Asia. In order to do this, we identified two DNA aptamers that bind and differentiate between small molecule partner drugs. These aptamers were selected from a library of single-stranded DNA molecules for their selectivity and binding affinity. Following their isolation by the capture-SELEX method, they were tagged with a fluorophore to visualize target binding upon release of a quenching DNA strand. Our aptamer sensors allow for the detection of piperaquine and mefloquine in the nanomolar range. We believe that this sensor will be a useful tool for monitoring and studying antimalarial drug resistance.

RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether

Abstract Authors	Yacheng Liao, Michael Fernandopulle, Guozhen Wang, Heejun Choi, Ling Hao, Rajan Patel, Catherine M Drerup, Seema Qamar, Jonathon Nixon-Abell, Yi Shen, William Meadows, Michele Vendruscolo, Tuomas Knowles, Matthew Nelson, Magda Czekalska, Greta Musteikyte, Christina Stephens, Amalia Pasolli, Lucy Forrest, Peter St. George-Hyslop, Jennifer Lippincott-Schwartz, Michael Emmerson Ward
Graduate Student Name	Michael Fernandopulle
NIH Institute-Center	NINDS
NIH Research Mentor	Michael Ward
UK Graduate University	Cambridge
University Research Supervisor	Peter St. George-Hyslop
Graduate Department/Program	Clinical Neurosciences/CIMR

Long-distance RNA transport plays a critical role in cells by enabling local protein translation at metabolically-active sites distant from the nucleus. This ensures an appropriate spatial organization of proteins, vital to polarized cells such as neurons. Here, we present a novel mechanism for RNA transport, in which RNA granules indirectly "hitchhike" on moving lysosomes. In vitro biophysical modeling, live-cell microscopy, and unbiased proteomics reveal that annexin A11 (ANXA11), an RNA granule-associated phosphoinositide-binding protein, acts as an adaptor between RNA granules and lysosomes. ANXA11 possesses an N-terminal low complexity domain, facilitating its phase separation into membraneless RNA granules, and a C-terminal membrane binding domain, enabling interactions with lysosomes. ALS-associated mutations in ANXA11 decrease long-range transport of RNA granules in neurons by disrupting their docking onto lysosomes. Thus, ANXA11 enables neuronal RNA transport via lysosomal hitchhiking of RNA granules, performing a critical cellular function that is disrupted in ALS.

Meningeal humoral immunity during homeostasis and neuroinfectious disease

Abstract Authors	Zachary Fitzpatrick, Kirsten Scott, Roger Barker, Dorian B. McGavern*, Menna Clatworthy*
Student Name	Zachary Fitzpatrick
NIH Institute-Center	NINDS
NIH Mentor	Dorian B. McGavern
UK University	Cambridge
University Supervisor	Menna Clatworthy/Roger Barker
University Department	Medicine

The central nervous system (CNS) was historically viewed as an immune-privileged site, segregated from the peripheral immune system. However, studies have demonstrated that the membranous lining of the CNS (or, meninges) is inhabited by a diverse repertoire of immune cells and that this barrier structure also acts as a gateway for CNS pathogens and neuroinflammation. In this study, we sought to characterize the meningeal humoral immune landscape under steady-state conditions and following systemic infection with Candida albicans - a human fungal pathogen known to invade the CNS.

Confocal microscopy of meningeal whole-mounts revealed both B cells and CD138+ BLIMP1+ plasma cells (terminally differentiated, antibody-secreting B cells) in naïve adult mice. Surprisingly, these plasma cells were predominantly IgA+, the antibody isotype found at mucosal surfaces, such as the gut. These meningeal IgA+ plasma cells were absent in germ-free mice but increased in number with age and following intestinal barrier breach. Spatially, IgA+ plasma cells localized adjacent to APRIL-expressing cells along the meningeal sinuses. Systemic candidiasis resulted in B cell infiltration and cluster formation 24 hours after infection, followed by increased IgA+ plasma cell numbers in the meninges.

This fungal pathogen was sequestered along the dural sinus wall early after infection and was found within B cell clusters. Local depletion of resident meningeal plasma cells enhanced mortality following intravenous candida challenge, which was associated with a reduction in fungal aggregates along the sinus wall. Our data demonstrate that the meninges house a substantial population of B lymphocytes and plasma cells, which are predominately IgA+. These cells localize along the dural sinuses, suggesting that the sinuses represent another important barrier structure (like the skin and gut) protected by local IgA. Meningeal IgA+ cells are dependent on the gut microbiome and appear to provide an early defense against pathogens introduced into the circulation, likely via promotion of pathogen entrapment.

On-going studies are focused on identifying the factors required for steady-state recruitment and retention of meningeal plasma cells as well as defining how these cells influence the dissemination of CNS pathogens.

Identification of Rasa3 as a novel PI3K effector protein in T cell adhesion and homeostasis

Abstract Authors	Kristoffer Haurum Johansen[1],[2],[3], Bonnie Huang1, Jennifer Cannons[1], Senta Kapnick[1], Fabien Garçon[2],[3], Klaus Okkenhaug[2],[3], Pamela Schwartzberg[1]
Graduate Student Name	Kristoffer Haurum Johansen
NIH Institute-Center	NIAID
NIH Research Mentor	Pamela Schwartzberg
UK Graduate University	University of Cambridge
University Research Supervisor	Klaus Okkenhaug
Graduate Department/Program	Department of Pathology

Integrin adhesion receptors play important roles in T cell activation, promoting interactions with antigen-presenting and other cells, migration, cytotoxicity and other effector functions. The major T cell integrin, LFA1, binds ICAM1, and is activated during TCR engagement through inside-out signaling. Studies suggest that inside-out signaling is mediated in part by phosphoinositide 3-kinase (PI3K), which phosphorylates the membrane lipid PI(4,5)P2 to PI(3,4,5)P3. However, the mechanism by which PI3K contributes to inside-out signaling remains unknown.

To evaluate roles of PIP3-binding proteins in inside-out signaling, we utilized CRISPR/Cas9 mutagenesis. Guide RNAs targeting PIP3-binding proteins highly expressed in T cells, were subcloned into retroviral vectors and used to infect murine T cells expressing Cas9. Transduction of T cells with gRNAs targeting PI3Kdelta led to ablation of PI3Kdelta and a reduction of LFA1-mediated ICAM1-binding. In contrast, knockout of PTEN, which increases PIP3 levels, increased ICAM1-binding. However, knocking out the PI3K effector, Akt, only minimally affected ICAM1-binding, suggesting other PIP3-binding proteins are involved in LFA1 activation. Screening of >120 gRNAs targeting 40 PIP3-binding and control proteins, identified proteins involved in LFA1-mediated adhesion, including Kindlin3 and Dynamin2. In contrast, KO of Rasa3, a Rap1GAP that has not been characterized in T cells, greatly increased ICAM1-binding and enhanced Rap1 activation. We further found that mouse models with mutations affecting Rasa3 activity show increased T cell counts with reduced activated T cells, altered homeostasis of T follicular helper cells, and increased adhesion and proliferation in vitro, suggesting an important and novel role for Rasa3 in T cell biology.

Intramural programs of NHGRI and NIAID, NIH, supported this work.

How to TRIAGE your screen hits: What Napoleon's doctor can teach us about big data interpretation and analysis

Abstract Authors	Samuel Katz, Jing Sun, Jian Song, Sinu John, Joseph Gillen, Nicolas Lounsbury, Ning Li, Aleksandra Nita-Lazar, Clare Bryant, Iain Fraser.
Graduate Student Name	Samuel Katz
NIH Institute-Center	NIAID
NIH Research Mentor	Iain Fraser
UK Graduate University	Cambridge
University Research Supervisor	Clare Bryant
Graduate Department/Program	Veterinary Medicine

The initiation of an immune response by inflammation is a central mechanism by which our immune systems activate their first line of defense. Yet, excessive and easily induced inflammation is a detrimental driving force in the pathogenesis and burden of many diseases. Consequently, inflammatory signaling cascades, such as the Toll-like Receptor (TLR) pathway, have had to develop complex regulatory mechanisms that prevent their activation by surmountable challenges while also rapidly initiating a sustained expression of the inflammatory gene program when sensing a robust danger signal.

To elucidate this regulatory mechanism we combined three high-throughput genome-scale approaches (gene perturbation screens, short-read and long-read RNA profiling, and proteomic analysis) and developed an iterative bioinformatic analysis model to facilitate the comprehensive identification of novel candidates in high-throughput studies. In the gene perturbation studies we find critical roles for proteasome-mediated protein degradation and stimulus-dependent RNA splicing to initiate and maintain the TLR-activated gene program.

While the degradation of negative regulators is known to be required for NF-kB and MAPK activation, we find an unexpectedly broad requirement for inhibitor degradation in all signaling branches downstream of TLR4, and identify master regulators of this process through proteomic analysis. Moreover, we establish a requirement for alternative RNA splicing to maintain sustained activation of TLR4 signaling components and characterize by complimentary RNAseq methods how the transcriptional repertoire of the cell is changed in response to TLR4 activation. We also use our genome-wide analysis to identify gene subnetworks among screen hits that link TLR activation to dysregulated splicing in myelodysplastic syndromes.

Our findings suggest a model whereby the TLR pathway activation threshold is enforced by broadly-acting negative regulators that are subject to proteasome-mediated degradation at a certain ligand dose, and that a transcriptional shift mediated by alternative splicing of critical TLR pathway components leads to sustained pathway activation after ligand challenge. These studies also demonstrate how a combination of systematic screening and computational analysis can identify important characteristics of innate immune responses across species, dissect distinct innate effector response pathways, and identify specific therapeutic targets for regulating TLR-driven outputs in disease states.

This work was supported by the Intramural Research Program of NIAID, NIH.

Microglia Suppress Tau Propagation in Late Stage Tauopathies

Abstract Authors	Hannah D. Mason, Ole Paulsen, Dorian B. McGavern
Graduate Student Name	Hannah Mason
NIH Institute-Center	NINDS
NIH Research Mentor	Dorian McGavern
UK Graduate University	University of Cambridge
University Research Supervisor	Ole Paulsen
Graduate Department/Program	Department of Physiology, Development, and Neuroscience

Dementia affects more than 45 million people worldwide with the number growing as the population continues to age. Alzheimer's disease and other tauopathies are some of the most common forms of dementia and are characterized by features such as tau (a microtubule-associated protein) aggregation, breakdown of CNS barriers, and neuronal loss. The leading risk factors for developing Alzheimer's disease, ApoE and TREM2, are linked to microglia, the brain's resident macrophage responsible for surveilling the environment and clearing cellular waste.

Previous studies in the P301S-humanized mouse model of tauopathy demonstrated that microglia depletion in the early stages of disease (3-4.5 months) significantly decreased tau propagation, leading to the hypothesis that microglia play a detrimental role in disease pathogenesis. To gain novel insights into how microglia contribute to the pathogenesis of tauopathies, we depleted microglia at a later time point (6 months) in humanized P301S mice. Microglia are known to fortify CNS barriers following damage, and our initial aim was to determine if microglia contributed to fortification of the glial limitans (an important CNS barrier) in P301S mice. We observed that microglia did in fact promote glial limitans integrity, as depletion resulted in increased barrier permeability. Unexpectedly, however, microglia depletion also caused a significant worsening of disease, which was characterized by increased cortical tau deposition, neurological dysfunction, and accelerated death.

There was also a strong positive correlation between neuronal tau loads in microglia-depleted mice and the degree of neurological dysfunction, suggesting that tau contributed to disease pathogenesis. These data conflict with published studies and suggest that microglia might play a time-dependent role in containing pathogenic tau during neurodegenerative diseases. We propose that microglia in later stages of a tauopathy help maintain CNS barriers and reduce pathogenic tau loads in neurons. Their absence or dysfunction at this time leads to enhanced tau spreading and death. Thus, it is important to consider that temporal aspects of microglia function when attempting to therapeutically manipulate these cells during tauopathies.

An orthotopic xenograft model for studying reirradiation and glioblastoma evolution

Abstract Authors	Joseph H. McAbee, Barbara H. Rath, Kristin Valdez, Dejauwne Young, Xiaolin Wu, Uma Shankavaram, Kevin Camphausen, Philip J. Tofilon
Graduate Student Name	Joseph Hiram McAbee
NIH Institute-Center	NCI
NIH Research Mentor	Dr. Philip J. Tofilon
UK Graduate University	Cambridge
University Research Supervisor	Dr. Colin Watts/Dr. Stephen Price
Graduate Department/Program	Clinical Neurosciences

Glioblastoma (GBM), the most common and malignant primary adult brain cancer, has a median survival of 15 months despite multimodal treatment involving surgical resection and chemo-radiotherapy. Ongoing clinical trials seek to examine the safety and benefit of retreatment with radiation therapy for recurrent glioblastoma. By adding a reirradiation protocol to a previously described glioma stem-like cell (GSC) initiated orthotopic xenograft model, this study seeks to better understand the impact of radiotherapy on both primary and recurrent GBM evolution and to establish an in vivo model for studying reirradiation. Therefore, we intracranially implanted CD133+ NSC11 cells, and other GSC lines, into nude mice. After 21 days, bioluminescence imaging was performed to confirm the presence of tumor prior to randomization into control and radiation therapy groups (3x5Gy). After treatment tumors were imaged weekly to track changes in BLI ratios. Once the average BLI ratio for the treated mice was found to be between 1 and 10, the mice were rerandomized into control (3x5Gy-Control) and radiation therapy groups (3x5Gy-3x5Gy). Following treatment, brain samples were collected at various time points out to morbidity to investigate changes in tumor morphology and histology.

Further, tumors from morbid mice were collected for viral integration site analysis (VISA), whole-exome sequencing (WES), and NanoString gene expression analysis. Survival analysis demonstrated a significant survival advantage for mice undergoing radiation therapy (+34.2 days) compared to controls. A further survival advantage was found for mice undergoing reirradiation (+30.0 days) compared to mice receiving only one course of radiation. On gross examination of morphology and H&E/SOX2 staining, brains bearing irradiated tumors and reirradiated tumors contained tumor tissue that was more likely to efface olfactory bulb(s) and less infiltrative than control tumors.

These histological changes were followed up with VISA which revealed that control tumors harbor fewer clones than in vitro lines and that irradiated tumors harbor the fewest clones of all. Gene Expression and IPA analyses showed that pathways involved in cell movement, survival, and proliferation are differentially regulated between irradiated and control tumors. WES was performed to compare gene mutation patterns between irradiated and control samples. Our results demonstrate that radiation, a central component of glioblastoma treatment, can have wide-ranging effects on the evolution of this dynamic tumor after initial presentation and recurrence. We have demonstrated for the first time the utility of a GSC-initiated orthotopic xenograft model for studying recurrent GBM biology and evolution. This reirradiation model may provide the opportunity to design and test more effective recurrent GBM treatment strategies that are centered around recurrent biology.

The characterization of DNA topoisomerase VI from *Methanosarcina mazei* using single molecule and ensemble methods

Abstract Authors	Shannon McKie
Graduate Student Name	Shannon McKie
NIH Institute-Center	NHLBI
NIH Research Mentor	Keir Neuman
UK Graduate University	University of East Anglia
University Research Supervisor	Anthony Maxwell
Graduate Department/Program	Wellcome trust scholar

Topoisomerase VI (topo VI) was the first discovered member of the type IIB topoisomerases and employs a double-stranded DNA break mechanism in order to alter the topology of the duplex. It is distinct from the type IIA topoisomerases such as prokaryotic DNA gyrase and topoisomerase VI and eukaryotic topoisomerase II, in regards to sequence and structure. Topo VI is an A2B2 heterotetramer, which can relax positive and negative supercoils, and decatenate and unknot DNA.

It is found principally in archaea, however has also been identified in plants and potentially plasmodia. The A subunit of topo VI is homologous to the meiotic recombination factor, Spo11, which is necessary for the formation of double-stranded DNA breaks during homologous recombination. In order to gain a deeper understanding of how topo VI functions, ensemble and single-molecule methods are being used to probe the mechanism of topo VI from *Methanosarcina mazei* (MmT6). This enzyme can be expressed in Escherichia coli and worked with at 37°C making it a tractable model for in depth characterisation. Any data collected on MmT6 can be translated into work done on the plasmodial and plant topo VI and may aid in finding antimalarial or herbicidal compounds

Visualization of Innate Immune Cell Dynamics During Early Stage Glioblastoma Development

Abstract Authors	Nicole Mihelson, Panagiotis Mastorakos, Fiona Powrie, Dorian B. McGavern
Graduate Student Name	Nicole Mihelson
NIH Institute-Center	NINDS
NIH Research Mentor	Dorian B. McGavern
UK Graduate University	University of Oxford
University Research Supervisor	Fiona Powrie
Graduate Department/Program	NDM: DPhil Biomedical Sciences: NIH-OH

Glioblastoma (GBM) is a universally fatal solid malignancy of the central nervous system, hallmarked by a high mitotic index, intercellular heterogeneity, and low neoantigen burden. Standard of care including surgery, radiation, and chemotherapy has failed to improve life expectancy beyond 15 months. The utility of local therapies is limited by the incredibly invasive nature of GBM, its protection behind the blood-brain barrier (BBB), and the absence of a single, druggable pathway.

In addition, GBM contains small populations of tumor cells that exhibit features attributed to stem cells. These features include the ability to self-renew and grow indefinitely, to differentiate into multiple lineages (i.e. multipotency) and most importantly to efficiently propagate tumor growth and resist cancer therapy. Advancing GBM therapies will likely require immune-based strategies given the ability of immune cells to traverse the BBB and evolve with the changing mutational landscape of the tumor. To this end, we have developed a murine GBM model that recapitulates the highly inflamed environment observed in the brain after surgical resection of the tumor.

Using intravital two-photon (2P) microscopy, we evaluated in real-time how individual tumor cells grow in the inflamed brain and reestablish a tumor mass – a scenario invariably faced by GBM patients following surgery. Our studies demonstrate that glioblastoma cells are incredibly resilient despite a sizeable post-surgical myelomonocytic response. Our long-term aim is to leverage this post-surgical inflammation against individual tumor cells and ultimately develop efficacious therapies that prevent GBM recurrence.

Defining the Molecular Mechanisms of Transcription-Associated Interstrand Crosslink Repair

Abstract Authors	William Nathan; Joseph Newman; Opher Gileadi; David Wilson III; Peter McHugh
Graduate Student Name	William Nathan
NIH Institute-Center	NIA
NIH Research Mentor	David Wilson III
UK Graduate University	Oxford University
University Research Supervisor	Peter McHugh
Graduate Department/Program	Weatherall Institute of Molecular Medicine

Interstrand crosslinks (ICLs) are DNA lesions that occur when a chemical agent covalently connects both strands of DNA. Generated by endogenous reactive species or exogenous agents, including many chemotherapeutics, ICLs can block the fundamental processes of DNA replication and RNA transcription. ICLs are processed in both replication-dependent and -independent manners, with the latter pathways playing a particularly critical role in post-mitotic cells. Previous work in the lab identified that SNM1A, a 5'-3' exonuclease and ICL repair protein, functionally and physically interacts with CSB, a DNA-dependent ATPase involved in transcription-coupled repair (TCR) and responsible for ~70% of cases of the premature aging disorder Cockayne syndrome (CS). This finding revealed that CSB plays a direct role in ICL repair and that ICLs may be repaired through a transcription-associated (TA) pathway, prompting the hypothesis that ICLs contribute to the CS clinical phenotypes and possibly also ordinary age-related disease.

To begin to elucidate the molecular mechanisms of TA-ICL repair, we have constructed an in-vitro system to partially reconstitute the processing of ICLs in the context of transcription. This effort has involved creating a novel DNA transcription bubble substrate containing a site-specific ICL and purifying the CSB, SNM1A, and ERCC1/XPF (an endonuclease complex) proteins. Our studies have uncovered that ERCC1/XPF cuts 5' to the transcription bubble on both strands of DNA, and SNM1A loads onto these sites and digests through the ICL in the 5'-3' direction, unhooking the lesion and resulting in a double strand break. In this work, we identified a novel functional interaction where ERCC1/XPF stimulates SNM1A exonuclease activity. CSB stimulates SNM1A exonuclease activity, but does not stimulate ERCC1/XPF endonuclease activity. The functional interaction between CSB and SNM1A is independent of CSB's ATPase activity. When all are present, CSB, SNM1A, and ERCC1/XPF can cooperate to process the ICL, possibly in an ATP-dependent manner. Based on our biochemical results, we propose a mechanism where CSB recognizes an ICL-stalled RNA polymerase and recruits the ERCC1/XPF and SNM1A nucleases. These nucleases then cooperate to unhook the lesion, creating a double-strand break which can be repaired through double-strand break repair pathways. This research provides new insights into replication-independent repair of ICLs and perhaps how these endogenous lesions may contribute to CS pathology and age-related disease.

Immunity and Memory Against Malaria: An Atlas of the Mosquito Immune System at Single-Cell Resolution

Abstract Authors	Gianmarco Raddi, Carolina Barillas-Mury, Oliver Billker
Graduate Student Name	Gianmarco Raddi
NIH Institute-Center	
NIH Research Mentor	
UK Graduate University	Cambridge
University Research Supervisor	
Graduate Department/Program	

Malaria is yearly responsible for 219 million cases and over 400,000 deaths. Anopheles gambiae mosquitoes are the main African vectors for the most virulent malaria parasite: *Plasmodium falciparum*. Mosquitos are not mere bystanders however, and rely on both humoral and cellular innate immune responses to defeat invading pathogens, including malaria.

These efforts are coordinated by hemocytes, the insect equivalent to vertebrate's white blood cells. Yet, hemocyte biology is largely unknown, mainly due to the low number and fragility of mosquito immune cells. In order to identify previously unknown cell types, their gene signatures, and their spatial-temporal localization in the mosquito we isolated Anopheles hemocytes and characterized them by single-cell RNA sequencing. A total of 5,218 individual Anopheles hemocytes were profiled 1,3 and 7 days after sugar-feeding, blood-feeding, or infection with Plasmodium berghei.

Ten cell sub-types were identified, including novel effector, inhibitory, phagocytic, and secretor cell subtypes. Bulk RNAseq of Anopheles hemocytes, guts, and carcasses was also performed. Genes that were both specific to each cell type in single cell RNA-seq data, and exclusively expressed in hemocytes in bulk RNAseq data were selected as cell markers. The putative cell types were validated with fluorescence in situ hybridization (RNA-FISH) in mosquito sections, whole guts and carcasses, and isolated hemocytes, showing an increase in active granulocytes and novel effector cells with malaria infection. After validation, challenged hemocytes' transcriptomic changes with time were investigated to understand hemocyte lineage and development. Both a rapidly dividing hemocyte progenitor pool and a trajectory of cell activation were identified, showing a progressive increase in immunity, signal transduction, spliceosome, and cell cycle genes from day 1 to 2 and 3, before returning to baseline at day 7.

Plasmodium infection leads to a dramatic increase of the novel secretor cell type. Our results are the first comprehensive transcriptomic study of a whole invertebrate organism's immune system, demonstrating hemocytes' complexity far exceeds what is currently described in the literature.

Impaired lysosome transport to distal axons contributes to autophagic stress in the neurodegenerative lysosomal storage disorder Niemann-Pick Type C

Abstract Authors	Joseph C. Roney, Tamar Farfel-Becker, Mei-Yao Lin, Xiu-Tang Cheng, Sean Cuddy, Frances M. Platt, Zu-Hang Sheng
Graduate Student Name	Joseph Roney
NIH Institute-Center	NINDS
NIH Research Mentor	Zu-Hang Sheng
UK Graduate University	University of Oxford
University Research Supervisor	Frances Platt
Graduate Department/Program	Department of Pharmacology

Niemann-Pick Type C (NPC) is a neurodegenerative lysosomal storage disorder characterized by accumulation of sphingosine, glycosphingolipids, sphingomyelin, and cholesterol in late endosomes and lysosomes. An early pathologic feature of NPC is axonal dystrophy, which consists of bulbous swellings along axons that contain accumulated organelles associated with the autophagy-lysosomal pathway. Such changes occur before symptom-onset and degeneration in NPC mice and suggest that defects in axonal organelle transport contribute to early NPC pathology. However, the mechanisms underlying these pathologic changes remain obscure.

Here we demonstrate that mature lysosome delivery to distal axons is significantly reduced in cortical neurons from Npc1 null mice, resulting in fewer numbers of lysosomes in NPC distal axons. Decreased axonal lysosome density leads to increased axonal autophagic stress that occurs without changes to autophagosome transport in NPC axons. This reduction in axonal lysosome density can be overcome by elevated expression of Arl8b, a small GTPase that mediates kinesin-1-dependent lysosome transport to the cell periphery. Rescuing axonal lysosome density by Arl8b expression reduces autophagic stress in axons from presymptomatic NPC mice. Collectively, these observations suggest that impaired lysosome transport to distal axons disrupts maturation and progression of the autophagy-lysosomal pathway and contributes to altered axonal homeostasis in NPC.

This work is supported by the Intramural Research Program of NINDS, NIH.

Posters

Structurally Conserved Bat IgG Antibodies Show a High Abundance of Sialylated Glycans: Potential Implications for Disease Resistance in Bats

Abstract Authors	Victoria A. Avanzato, Yasunori Watanabe, Alice Stelfox, Steph Seifert, Tony Schountz, Karl Harlos, Tom Walter, Max Crispin, Vincent Munster, Thomas A. Bowden
Graduate Student Name	Victoria Avanzato
NIH Institute-Center	NIAID
NIH Research Mentor	Vincent Munster
UK Graduate University	Oxford
University Research Supervisor	Thomas Bowden
Graduate Department/Program	Division of Structural Biology

The prototypic henipaviruses, Nipah and Hendra, are emergent paramyxoviruses capable of causing highly fatal outbreaks associated with extreme neurological and respiratory disease. Despite the severe disease caused in spillover hosts, reservoir host bats do not show negative clinical symptoms in response to natural or experimental infection. This striking difference in disease pathology raises questions about how features of the bat immune system differ from those of humans in the context of control of viral replication and clinical outcome, and presents the need to further classify the immune components of the host species in detail.

This project aims to provide a structural basis for the host response to henipavirus infection by characterizing the antibodies of reservoir bats, particularly the Fc region, using a crystallography approach. Although bats are the primary reservoir for several viral diseases, this is the first in-depth analysis of bat Fc proteins. The Fc region of IgG is involved in antiviral immunity via binding to Fc receptors, which either activates or inhibits immune effector cells, depending on the receptor subtype and the presence and composition of N-linked glycans on the Fc molecule. The IgG Fc region from Pteropus alecto, a confirmed host for Hendra virus, was expressed and crystallized individually as well as bound to the bat high affinity Fc receptor, FcyRI. Analysis of multiple crystal forms of the P. alecto Fc showed an overall conserved structure and mechanism for receptor binding to human Fc. A conserved feature of the binding site is a hydrophobic pocket on FcyRI into which a specific leucine residue on the Fc molecule is inserted. This contact is critical for the high affinity of the FcyRI – IgG interaction in the human complex, and it appears that this mechanism for high affinity binding is conserved in P. alecto.

In addition, as the Fc glycans are crucial to Fc effector function, we sought to analyze the native Fc glycan composition of IgG purified from bat serum using Ultra Performance Liquid Chromatography (UPLC). Unexpectedly, the bat serum IgG displayed an abundance of sialylated glycans on both the Fc and the Fab regions, which is not typically seen on human antibodies (the level of sialylated IgG is ~10% in human serum). As sialylated antibodies are associated with an anti-inflammatory immune profile, their apparent preponderance on bat IgG may have implications for how the bat immune system responds to and tolerates viral infection.

Neurons rely on endosomal autophagy for protein turnover

Abstract Authors	Benjamin Badger, Andrea Brand
Graduate Student Name	Benjamin Badger
NIH Institute-Center	NINDS, NHLBI
NIH Research Mentor	Richard Youle
UK Graduate University	Cambridge
University Research Supervisor	Andrea Brand
Graduate Department/Program	Department of Physiology, Development, and Neuroscience

Autophagy is the process by which cytosolic material is sent to the lysosome for destruction and is necessary to prevent the formation of ubiquitinated protein aggregates that lead to neurodegeneration. We investigated the mechanism of autophagy in larval Drosophila neurons and find that these cells have a very high amount of basal autophagic flux at the cell body but not in axons.

Neuronal somas require Atg1 but not the essential conventional macroautophagic proteins Atg5 or Atg12 for most autophagic flux. Neuronal somas do not require Snap29-mediated autophagosome-lysosome fusion, in contrast to what is observed for neural stem cells and neuronal axons. Snf7 in ESCRT III is found to be necessary for neuronal soma as well as neural stem cell autophagy, and depletion of this protein leads to the accumulation of nonacidified autophagic substrates.

These autophagic substrates are found surrounded by plasma membrane-targeted protein in neuronal somas, but are mostly surrounded by ER-targeted protein in neural stem cells. These observations suggest that neuronal endosomal membranes take up cytosolic material in an ATG1-dependent but ATG-independent manner, consistent with previous reports of endosomal microautophagy as well as alternative macroautophagy. Consistent with the hypothesis that endosomal microautophagy plays a role in uptake of cytosol into endosomes of neuronal somas, we find that a minority of early endosome-targeted YFP-RAB5 vesicles contain autophagic material. Here we present evidence towards the idea that neurons require endosomal micro- and perhaps macro- autophagy for protein turnover to prevent p62-bound aggregate formation.

Real-time deformability cytometry reveals the biophysical kinetics of neutrophil immune function, particularly in the context of lupus

Abstract Authors	Kathleen Bashant, Nicole Toepfner, Arlette Vassallo, Cecilia Matara, Natasha Jordan, Nehal N Mehta, Edwin R Chilvers, Charlotte Summers, Mariana J Kaplan
Graduate Student Name	Kathleen Bashant
NIH Institute-Center	NIAMS
NIH Research Mentor	Dr. Mariana Kaplan
UK Graduate University	University of Cambridge
University Research Supervisor	Dr. Charlotte Summers
Graduate Department/Program	Medicine

It has become increasingly apparent that the biophysical properties of neutrophils impact their trafficking through the circulation, particularly through the pulmonary capillary bed.

For example, the retention of shape-changed neutrophils in the lungs was recently proposed to contribute to acute respiratory distress syndrome pathogenesis. Accordingly, this study tested the hypothesis that neutrophil priming is coupled to biophysical changes capable of altering cell immunologic function.

We employ real-time deformability cytometry (RT-DC), a recently developed, rapid, and sensitive method to assess the distribution of size, shape, and deformability of thousands of cells within seconds. During RT-DC analysis, neutrophils can be easily identified within anticoagulated blood based on their unique granularity and size, thus avoiding the need for further isolation techniques.

Hence, RT-DC is uniquely suited to describe the kinetics of biophysical cell changes in an in vivo relevant manner. We reveal that following priming, neutrophils undergo a short period of cell shrinking and stiffening, followed by a phase of cell expansion and softening. In some contexts, neutrophils ultimately recover their un-primed mechanical phenotype, exhibiting a “de-priming” phenomenon.

The mechanism(s) underlying changes in human neutrophil size are shown to be Na+/H+ antiport-dependent, specifically through induction of macropinocytosis. Furthermore, RT-DC reveals that neutrophils found in the blood of patients with active systemic lupus erythematosus (SLE) or respiratory viral infection phenotypically resemble primed neutrophils. Low density granulocytes (LDG), a subset of neutrophils found in patients with SLE, are also shape-changed and yet appear biophysically distinct from both unprimed and primed normal dense neutrophils. Current research efforts are aimed at unraveling the interplay between this new knowledge of SLE neutrophil biomechanics and cell immunologic function.

Proposed intermittent ion channel trafficking presents speed-stability tradeoff around transport bottlenecks

Abstract Authors	Adriano Bellotti, Dax Hoffman, Tim O'Leary
Graduate Student Name	Adriano Bellotti
NIH Institute-Center	NICHD
NIH Research Mentor	Dax Hoffman
UK Graduate University	Univ of Cambridge
University Research Supervisor	Tim O'Leary
Graduate Department/Program	Engineering

Neurons are excitable cells that contain processes that can extend hundreds or thousands of microns away from the cell body. This unique morphology presents several logistical obstacles regarding the function of the cell as a cohesive unit. One such challenge is the transport and regulation of ion channel concentrations throughout the dendritic arborization of pyramidal cells. To compound the issue, Kv4.2 is a voltage-gated ion channel that is endogenously expressed with a particularly demanding profile showing increasing density with distance from the soma. Previous modeling studies confirm that such cargo demand profiles create transport bottlenecks and result in critical speed-precision tradeoffs.

We propose that ion channels such as Kv4.2 are trafficked intermittently in discrete on/off states to alleviate transport bottlenecks. This hypothesis is based on 100+ hours of dendrite recording following transfection of Kv4.2-GFP. Microtubule-based active transport was first confirmed using colchicine drug trials. Dendritic transport was observed in only 30 percent of total recording time, and dendritic activity appears to follow a dichotomous random process with puncta appearing in clustered bursts. Interestingly, axonal trafficking is present during 90 percent of total recording time, and puncta transport in axons appear to be continuous with no distinct off-state. Inferential statistical analysis of the dataset is performed to determine which stochastic process best describes the underlying intermittent phenomenon. Prospective models include the inhomogenous Poisson point process, the telegraph process, and combinations of these processes.

The observed intermittent phenomenon has been replicated in silico to assess its feasibility in relieving transport bottlenecks and improving system stability. Initial open-loop simulations reveal a speed-stability tradeoff governed by the frequency of the intermittent phenomenon. Closed-loop simulations were then performed with linear and nonlinear controllers. Linear controllers with increased gains result in instability, whereas the on-state of an on/off nonlinear switch controller can be amplified without instability. Further, the on-state in the nonlinear controller occurs intermittently over time, resembling the on/off states observed in vitro. Complex analysis of poles and zeros is required to better understand the nonlinear system.

Modeling chromosome instability in ovarian cancer.

Abstract Authors	Daniel Bronder[1],[2], Thomas Ried[2], Stephen Taylor[1]
	[1]The University of Manchester, [2]National Cancer Institute
Graduate Student Name	Daniel Bronder
NIH Institute-Center	
NIH Research Mentor	
UK Graduate University	
University Research Supervisor	
Graduate Department/Program	

High-grade Serous Ovarian Cancers (HGSOC) are characterized by high levels of chromosome instability (CIN) and resulting chromosomal copy number aberrations (aneuploidy). Consequences of CIN in cancer cells are intratumoral genetic heterogeneity, cancer genome evolution and therapy resistance. CIN can be driven by various mechanisms including chromosome missegregation in mitosis and replication stress in interphase. High levels of aneuploidy in cancer genomes have been correlated with frequent mutations in the tumor suppressor gene TP53, which is mutated at a very high frequency of 96% in HGSOC.

Here, we aim to mimic HGSOC-characterizing mutations to assess their impact on CIN in the early stages of high-grade serous ovarian carcinogenesis using a human cell line (FNE1) derived from the fallopian tube as it has been suggested as the tissue of origin of HGSOC. Firstly, we set out to characterize FNE1 cells confirming tissue origin and genome stability to ensure their use as a good model system. Upon characterization of the FNE1 cells, we aimed to knock out tumor suppressor genes and overexpress oncogenes using CRISPR/Cas9 technology in combination with lentiviral vectors.

The initial characterization of the human, non-transformed, hTERT-immortalized cell line FNE1 by immunofluorescence and flow cytometry has confirmed its fallopian tube origin. In addition, we validated wild-type TP53 status and function by Sanger sequencing and immunoblotting respectively. Moreover, karyotyping has revealed a stable, near-diploid karyotype with a clonal translocation between the short arm of chromosome 9 and the long arm of chromosome 15. Then, using lentiviral transduction, FNE1 cells expressing tetracycline-inducible Cas9 have been generated and utilized to knock out the tumor suppressor genes TP53 and BRCA1. In addition, doubly and triply transgenic FNE1 cells overexpressing the oncogene MYC have been created. Using a small molecule inhibitor of Cenp-E to challenge mitotic fidelity in FNE1 cells, we show that p53 loss confers tolerance to mitotic stress.

Future experiments aim to analyze the wild-type and mutant cells' transcriptome and tumorigenic potential by RNA sequencing and injection into the ovarian bursa of nude mice, respectively. Multiplex interphase Fluorescence in situ Hybridization (miFISH) will be utilized to assess changes in copy number of genes frequently gained and lost in HGSOC.

Single-cell resolution of metabolic control over HIV-1 entry and a role for membrane lipid order and tension

Abstract Authors	Charles A. Coomer, Maro Iliopoulou, Michael L. Dustin, Ewaldus B. Compeer Alex A. Compton and Sergi Padilla-Parra
Graduate Student Name	Charles Coomer
NIH Institute-Center	National Cancer Institute
NIH Research Mentor	Alex Compton
UK Graduate University	University of Oxford
University Research Supervisor	Sergi Padilla-Parra, Ewoud Compeer
Graduate Department/Program	NIH Oxford-Cambridge Scholars Program, Nuffield Department of Medicine, Wellcome Trust Centre Human Genetics

Recent studies have highlighted cellular metabolic activity as a critical factor driving HIV-1 infection in T cells. However, deciphering how the metabolic state of single cells affects virus entry remains to be fully characterised.

We developed an assay utilising FRET-based biosensors of various metabolites to evaluate the influence of global metabolic processes on the success rate of virus entry in single cells. Lifetime fluorescence images of single cells were recorded immediately before and after addition of HIV-1 pseudovirions (i.e. HIV-1JR-FL) or non-enveloped HIV-1 with incorporated BLaM-Vpr.

Lifetime measurements of cells expressing biosensors for ATP:ADP ratio or lactate were utilised to determine relative metabolite concentrations before and during entry. The same cells were subsequently screened for fusion and productive infection to determine whether baseline intracellular metabolite concentrations were correlated with these processes.

Interestingly, cells with a lower ATP:ADP ratio prior to virus addition were less permissive to virus fusion and infection. These results indicated a relationship between host metabolic state and the likelihood for virus-cell fusion to occur. To confirm this, we show that cells treated acutely with 2-deoxy-d-glucose (2-DG), an inhibitor of glycolysis, permitted substantially fewer fusion events. Single particle tracking (SPT) revealed that virions were arrested at hemifusion in 2-DG-treated cells. Interestingly, cells treated with 2-DG also possessed less surface membrane cholesterol, while the addition of cholesterol to the plasma membrane rescued the block to fusion. Further investigation with additional reporters revealed a link between host glycolytic activity and membrane tension and order, with cells treated with 2-DG exhibiting lower plasma membrane lipid order and higher tension values.

These data suggest that low glycolytic activity results in a deficiency of membrane cholesterol. Finally, SPT illustrated that virions were less likely to enter cells at areas of high membrane tension. We are currently performing similar experiments in T cells. We have identified a connection between host glycolytic activity and membrane tension which may influence HIV-1 fusion at the single-cell level. Our results indicate that HIV-1 fuses with glycolytically-active cells and that this activity is linked to cell surface membrane cholesterol and membrane tension.

Fc Receptor of IgA and IgM is a Novel Candidate Gene for Primary Immunodefiency

Abstract Authors	Madeline Epping, Pamela Schwartzberg, Ken Smith
Graduate Student Name	Madeline Epping
NIH Institute-Center	NIAID
NIH Research Mentor	Pamela Schwartzberg
UK Graduate University	Cambridge
University Research Supervisor	Ken Smith
Graduate Department/Program	Medicine

Primary immunodeficiency disorders (PID) are group of over 350 rare, genetic conditions affecting the development or function of the immune system. The most severe forms typically present early in childhood but the majority of PID patients emerge in adulthood with variable clinical symptoms suggesting immune dysregulation. These patients often lack a clear family history and it remains difficult to decipher the underlying genetic cause of sporadic cases. PID patients classically display clinical symptoms spanning immunodeficiency, autoimmune conditions, and cancer. Deciphering the underlying mechanisms of PID can provide a better understanding of the immune dysregulation present in a wide range of disorders.

Given the highly variable nature of PID and often overlapping clinical definitions of PID subtypes, a cohort approach was taken to identify novel gene candidates responsible for immunodeficiency across PID as a whole. The NIHR BioResource Rare Diseases program has compiled a GWAS cohort of 974 sporadic and familial PID patients with 344 unaffected relatives and an additional 9,283 unrelated controls. BeviMed analysis confirmed a number of known PID genes and provided a set of novel candidates that have not been previously associated with the condition.

Fc Fragment of IgA and IgM Receptor (FCAMR) was identified as a candidate gene in the screen and was selected for further analysis. Patients containing heterozygous variants were found to portray a late-onset common variable immunodeficiency-like phenotype, with antibody deficiency and autoimmune conditions. FCAMR has its highest expression in follicular dendritic cells and previous work in murine models suggest that the protein suppresses germinal center formation and memory B-cell generation, as well as increases autoantibody production. However, the structure and expression patterns of the human and murine FCAMR differ and it remains to be determined what the functional role is in humans. Initial studies will assess RNA and protein expression in variant-containing patients and controls, as well as explore localization defects and splicing alterations. Subsequent studies will address immunoglobulin binding capacity, cytokine production, and signaling defects in cells containing knock-out and variant forms of FCAMR.

This work could determine the function of a largely unknown Fc receptor and its role in maintaining immune homeostasis, including clonal selection of B cells, capacity to respond to infections, and mechanisms of IgA and IgM associated disease suppression. This can be applied to the broader immune dysregulation experienced in PID and can offer insight into the elements that tip the balance to autoimmunity and immunodeficiency. Ideally, this work will identify new areas for targeted therapy for a range of immune-mediated disorders.

Tracking Non-Classical Immune Responses to the Microbiome in Humans

Abstract Authors	Taylor Farley[1], Federico Mele[2], Siddharth Krishnamurthy[1], Oliver Harrison[1], Ai Ing Lim[1], Federica Sallusto[2], Fiona Powrie[3], Yasmine Belkaid[1]
Graduate Student Name	Taylor Farley
NIH Institute-Center	NIAID
NIH Research Mentor	Yasmine Belkaid
UK Graduate University	Oxford
University Research Supervisor	Fiona Powrie
Graduate Department/Program	Nuffield Department of Medicine

Inflammatory bowel disease (IBD) encompasses a group of disorders affecting both the large and small intestine. Although they differ in location throughout the gastrointestinal tract, these afflictions are united by destruction in the epithelial barrier of the gut and aberrant immune responses to the microbiome. Studies have investigated classical immune responses in IBD, largely focusing on the role of CD4+ T cells. However, the intestinal epithelium is densely populated with CD8+ T cells. While transcriptional analysis of circulating CD8+ T cells in patients with IBD can predict clinical outcomes, the role and antigen specificity of mucosal tissue resident CD8+ T cells remains unclear.

The epithelial layer of the gut provides a vital barrier between host and the trillions of bacteria, viruses, fungi and protozoa that are collectively termed the "microbiota". Barrier sites such as the gut are enriched with cells utilizing non-classical MHC presentation including CD8+ T cells. Recently in mice, we demonstrated that non-classical MHC recognition of the microbiota could control tissue physiology. Notably, we showed novel means of maintaining the epithelial barrier of the skin dominated by CD8+ T cell responses to microbial N-formylated (fMet) peptides presented by a non-classical MHC molecule, H2-M3. Our more recent work describes a novel human functional homologue of H2-M3, also capable of presenting fMet peptides to CD8+ T cells.

We therefore hypothesize that non-classical presentation of microbial fMet peptides to CD8+ T cells in the gut could contribute to the maintenance of the intestinal barrier and would be perturbed in the inflammatory environment of IBD. To address this hypothesis, we have developed human tetramer reagents to track and isolate microbiota derived N-formylated antigen specific T cells from the blood and tissues of patients, measure their frequency and probe their function ex vivo by single cell RNA sequencing. Additionally, we will dive deeper into the functional consequences of these homeostatic or deranged responses, by utilizing mouse models of intestinal inflammation. This project ultimately aims at uncovering dominant mechanisms associated with the recognition of commensal derived antigens with the goal of developing novel approaches to restore host-microbe homeostasis in the context of IBD.

Towards engineered autologous cellular therapies for RAG2 deficiency

Abstract Authors	Cameron Lee Gardner[1],[2],[3], Mara Pavel-Dinu[4], Marita Bosticardo[1], Suk See DeRavin[1], Harry Malech[1], Matthew Porteus[4], Oreste Acuto[2], Luigi Notarangelo[1] [1]Laboratory of Clinical Immunology and Microbiology NIAID National Institutes of Health, [2] Sir William Dunn School of Pathology, University of Oxford [3] Nuffield Department of Medicine, University of Oxford [4] Department of Pediatrics, Stanford University, Stanford
Graduate Student Name	Cameron Gardner
NIH Institute-Center	NIAID
NIH Research Mentor	Dr. Luigi Notarangelo
UK Graduate University	University of Oxford
University Research Supervisor	Prof. Oreste Acuto
Graduate Department/Program	NDM, Sir William Dunn School of Pathology

The future of personalized medicine is shifting towards gene and cellular therapies. One prime candidate gene for generation of a novel cellular therapy target is for recombination activating gene 2 (RAG2). RAG2 is essential in lymphocyte development across many species, and without a functional copy of the gene humans develop severe combined immunodeficiency (SCID), an extreme lymphocytopenia condition wherein patients have virtually zero B or T cells and are therefore very susceptible to infection. The current standard of care is a bone marrow transplant (BMT), however even matched BMTs come with a host of complications including possible graft versus host disease and incomplete and failed immune reconstitution. We present here a strategy for taking an ex vivo approach to gene correct patient hematopoietic stem cells to be used in an autologous stem cell transfer, thus ameliorating many of the complications arising from a traditional BMT. Rescue of development of edited patient cells was examined using an artificial thymic organoid system.

Clathrin-dependent endocytosis is a LRRK2-mediated, Parkinson's disease relevant pathway

Abstract Authors	George Heaton, Adamantios Mamais, Natalie Landeck, Ravindran Kumaran, Laura Pellegrini, Alexandra Beilina, Michael Nalls, Yan Li, Kirsten Harvey, Mark R. Cookson
Graduate Student Name	George Heaton
NIH Institute-Center	NIA
NIH Research Mentor	Mark Cookson
UK Graduate University	UCL
University Research Supervisor	Kirsten Harvey
Graduate Department/Program	NIH-Wellcome trust

Mutations in the LRRK2 gene represent a major cause of Parkinson's disease (PD), a common age dependent neurodegenerative disease characterised by nigral neuronal loss and motor symptoms. LRRK2 is a pleiomorphic locus as common PD-associated variants have been identified through genome-wide association studies (GWAS) as well as in large familial case-studies. Despite intensive study into the physiological function of LRRK2, a definitive consensus on how LRRK2 acts within cells and how pathogenic mutations compromise this function has yet to be achieved.

Understanding protein-protein interactors of LRRK2 can offer key insight into cellular mechanisms leading to neurodegeneration. In this study, we employed sequential screens to identify direct pathogenic modifiers of LRRK2 function. We report a functional interaction between LRRK2 and Clathrin adaptor protein complex 2 (AP2). This interaction was found to be conserved in Brain and Kidney, with KO animals having significant dysregulation of AP2 and Clathrin.

We hypothesized that LRRK2 was capable of regulating Clathrin dependent endocytosis (CDE), a core function of the AP2 complex. We demonstrate that LRRK2 expression is capable of inducing defects in CDE in a mutational dependent manner. Consistent with defects in Clathrin associated trafficking, we observed decreased activity-dependent synaptic vesicle endocytosis within mutant LRRK2 knock-in neurons.

Given the convergence of LRRK2 and several PD-associated genes within the endocytic pathway, we used polygenetic analysis to probe for risk-association. SNPs linked with Clathrin-dependent endocytosis genes were found to be associated with Parkinson's disease across multiple cohorts, suggesting common variants at these loci represent a major risk factor for disease.

Phenotypic Screening and Functional Genetics in FTD/ALS

Abstract Authors	Stewart W. Humble[1],[2],[3], Michael E. Ward[1], Richard Wade-Martins[2]
	1. Inherited Neurodegenerative Diseases Unit, Neurogenetics Branch, NINDS, National Institutes of Health; 2. Wade-Martins Group, Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics, Medical Sciences Division, University of Oxford; 3. Louisiana State University Health Sciences Center New Orleans, School of Medicine
Graduate Student Name	Stewart W. Humble
NIH Institute-Center	NINDS
NIH Research Mentor	Dr. Michael E. Ward
UK Graduate University	University of Oxford
University Research Supervisor	Prof. Richard Wade-Martins
Graduate Department/Program	NDM, Department of Physiology, Anatomy, and Genetics

Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are adult-onset neurodegenerative diseases that are closely related clinically, pathologically, and genetically. Therefore, it is likely that converging disease pathways exist and can be used both to enhance our understanding of basic FTD/ALS biology and as leads for therapeutic intervention. FTD/ALS is often inherited and causal mutations are highly penetrant, indicating that these mutations have large effects on critical cellular pathways. Approximately half of the genes known to cause FTD/ALS encode proteins that regulate endolysosomal biology or play a crucial role in regulating proteostasis. Our own unpublished data implicates lysosomal dysfunction as a central pathophysiological consequence of mutations in GRN due to defects in endolysosomal acidification and/or maturation, building upon previously established work from my mentor demonstrating that lysosomes are a key player in FTD/ALS-related neurodegeneration.

Here, we propose to use our modified i3Neuron platform to facilitate systematic knockdown of large panels of FTD/ALS-associated genes through CRISPR interference (CRISPRi) to interrogate Gene-Drug and Gene-Gene interactions. From this, we will generate genetic interaction maps of pairs of FTD/ALS genes in iPSC-derived neurons, identifying common pathogenic changes elicited by disease genes related to several key cellular processes, including endolysosomal acidification and maturation, lysosomal ion homeostasis, selective autophagy, organelle quality control, and neuronal survival. We will also use drug libraries with well-characterized molecular targets in combination with CRISPRi knockdowns to more precisely define sensitized pathways in FTD/ALS. Through these efforts, we hope to lay the groundwork for improved molecular understanding and therapeutic discovery.

Somatic mutation landscape of tissue resident lymphocytes in Primary Sjogren's Syndrome

Abstract Authors	Aleksandra Ivovic, Paul Milne, Heather Machado, Fai Ng, Emmanuella Traianos, Richard Siegel, Matthew Collin, Peter Campbell
Graduate Student Name	Aleksandra Ivovic
NIH Institute-Center	NIAMS
NIH Research Mentor	Richard Siegel
UK Graduate University	Cambridge
University Research Supervisor	Peter Campbell
Graduate Department/Program	Sanger Institute (Cancer, Ageing, and Somatic Mutation)

In recent years, interest in somatic mutations has expanded beyond the scope of cancer into investigations of normal tissue and a range of nonmalignant pathologies. Perhaps contrary to expectation, oncogenic driver mutations can be found in normal tissue and benign conditions. Mutational signatures and overall burden can be used to infer exposure of cell populations to various environments and mutagens.

We are interested in investigating the occurrence of somatic mutations in autoimmune diseases such as Primary Sjogren's Syndrome (PSS) and examining whether resident lymphocytes in affected tissues carry an altered mutational profile. Our aim is to understand whether somatic mutations drive clonal expansion of autoreactive lymphocytes. We have obtained samples from diagnostic salivary gland biopsies from PSS patients and analyzed them with targeted sequencing, whole genome sequencing, and single cell sequencing.

Preliminary targeted sequencing of FACS-sorted T and B cell compartments shows a diverse population of lymphocytes present in aggregates in the salivary gland. B and T cells generally display receptor polyclonality and diverse subclonal variation. Interestingly, we found clonal expansions containing truncating mutations in the KDM6A tumor suppressor gene in CD8+ T cell compartments of two patients, which we will examine with further sequencing studies.

To isolate histological features of the biopsies including lymphoid aggregates, glands, and ducts, we employed laser capture microdissection followed by targeted and whole genome sequencing. Additionally, we will perform single cell transcriptome and B cell repertoire sequencing to assess levels of gene expression in conjunction with BCR clonotype. Here we present initial interpretations of these results.

Analysis of Parkinson's disease at a single neuron level

Abstract Authors	Mindaugas Jonikas, Michael Ward, Pawan Kumar, Richard Wade- Martins
Graduate Student Name	Mindaugas Jonikas
NIH Institute-Center	Inherited Neurodegenerative disease unit
NIH Research Mentor	Michael Ward
UK Graduate University	Richard Wade-Martins & Pawan Kumar
University Research Supervisor	University of Oxford
Graduate Department/Program	Department of Physiology, Anatomy and Genetics

Parkinson's disease is the second most common neurodegenerative disease worldwide. However, no available therapies alter the underlying neurodegenerative process, characterized by loss of dopamine neurons in the substantia nigra pars compacta and subsequent degeneration of cortical neurons, with widespread α-synuclein aggregation.

Stem cell differentiation methods progress rapidly, creating good models where we can obtain human dopamine and cortical neurons from induced pluripotent stem cells derived from patients with mutations in the α-synuclein gene. These models allow exploration of the effects of the mutations in the α-synuclein gene whilst retaining the patient's genetic background. We have developed versatile and scalable dopaminergic and cortical neuron differentiation protocols that successfully produced electrophysiologically active neurons carrying mutations in the SNCA gene (A53T and triplication of SNCA), suitable for single neuron high-throughput image analysis.

We have tested, adapted and implemented the image based Cell-Painting assay (Bray et al., 2017), which allow us to quantify multiple molecular and phenotypic changes of stem cell derived neurons with mutations in the α- synuclein gene (A53T and triplication of SNCA). Firstly, we demonstrated that Cell-Painting assay is very sensitive analysis method, which is able to detect fine differences between toxin treatments. Secondly, we developed data profiling tools to cluster treated cells and extract biological reasons for clustering of the cells.

Thirdly, we applied these methods to analyse stem cells derived dopaminergic neurons with mutations in SNCA gene (A53T and triplication of SNCA), where found the distinct differences between neurons with and without mutation, as well as between these mutation. Lastly, by comparing observed phenotypes with published databases, we selected and screened compounds, which have opposite effect to the phenotypes observed in dopaminergic neurons having A53T mutation and triplication of SNCA. Some of the compounds have reversed differences observed between "heathy controls" neurons and A53T mutation and triplication of SNCA, therefore we are currently extending screening by selecting even more specific compounds.

In this work, we show how to apply high-throughput microscopy to reveal significant differences and delineate fine changes at single neurons. In addition, we developed tools allowing us to manipulate and reverse those difference. This is a novel, non-biased and non-specific Parkinson's disease analysis approach, which revealed novel drug targets and potentials compounds to modulate observed differences!

Understanding the roles of PTEN and DNA Damage Response in the Treatment Response of Metastatic Prostate and Breast Cancers

Abstract Authors	Emily Kolyvas, Carlos Caldas, Kathy Kelly
Graduate Student Name	Emily Kolyvas
NIH Institute-Center	NCI
NIH Research Mentor	Kathy Kelly
UK Graduate University	Cambridge
University Research Supervisor	Carlos Caldas
Graduate Department/Program	CRUK

Genomic instability is a hallmark of cancer. Defects in the DNA damage response (DDR) can allow for DNA mutations that lead to cancer, however, these defects can also be used as therapeutic targets. DDR inhibitors are currently being developed and tested in the clinic for a range of malignancies. This approach exploits replication stress as a major cause of DNA damage, and targets proteins that are important in the replication stress response, such as ATR, ATM and WEE1.

Recent data from the Kelly lab has shown that PTEN null patient derived xenograft (PDX) prostate cancer organoids have increased sensitivity to some DDR inhibitors over PTEN WT organoids. The goal of the project proposed here is to understand the mechanism of the relationships between PTEN status, DDR phenotype, and sensitivity to DDR inhibitors. To address these aims, the DDR phenotypes of PTEN null prostate cancer PDXs will be characterized following exogenously induced replication stress and following treatment with cell cycle checkpoint inhibitors.

Further, this study will aim to understand the mechanistic role of PTEN in DDR and how it relates in various PTEN null cancer models, including prostate and breast cancers. These studies will help to elucidate the mechanism of increased sensitivity to DDR inhibitors, investigate PTEN mutational states as a treatment biomarker, and provide insight into the potential for translation of novel therapies for PTEN null cancers, including metastatic prostate cancer and breast cancer.

7T fMRI-fMRS: Investigating measures of BOLD and neurochemical concentrations across time in the human motor cortex

Abstract Authors	Jacob M. Levenstein[1,2], William T. Clarke[1], Uzay E. Emir[1,3], Peter Bandettini[2], Charlotte J. Stagg[1]
	Affiliations: 1. Wellcome Centre for Integrative Neuroimaging, FMRIB, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, OX3 9DU, UK 2. Section of Functional Imaging Methods, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA 3. School of Health Sciences, Purdue University, West Lafayette, IN, USA
Graduate Student Name	Jacob M. Levenstein
NIH Institute-Center	National Institutes of Mental Health
NIH Research Mentor	Dr. Peter Bandettini
UK Graduate University	University of Oxford
University Research Supervisor	Professor Charlotte Stagg
Graduate Department/ Program	Nuffield Department of Clinical Neuroscience, Wellcome Centre for Integrative Neuroimaging

Functional magnetic resonance spectroscopy (fMRS) is an in-vivo brain imaging technique that shows promise towards measuring task-relevant modulation of neurotransmitters in the human brain. Despite early work demonstrating the utility of proton MRS (1H-MRS) to observe changes in MRS spectra collected during rest and during visual stimulation (Prichard, et al., 1991), the number of published fMRS studies are low, with less than twenty-five focused on measuring glutamate, a major excitatory neurotransmitter with a key role in energetic processes (Mullins, 2018). Ip et al. (2017) is one such example, though their work is of particular value, as their fMRS sequence includes an interleaved measure of blood-oxygen level dependent (BOLD) contrast imaging.

BOLD imaging, also known as fMRI, is a systems level MRI technique used to measure changes in blood oxygenation and infer activity in brain regions across time. Ip et al. (2017) find a task relevant positive correlation between group-level fMRI timecourses in the visual cortex and fMRS glutamate. These results suggest that fMRS can detect task relevant group-level changes in glutamate over time. Further, these results also suggest that glutamate could be an additional mechanism underlying the observed systems level change measured with fMRI.

Using a modified version of the combined fMRI-fMRS sequence employed in Ip et al. (2017), we hypothesized that spectral data collected from M1 during a motor task would result in a positive task relevent BOLD-glutamate timecourse correlation. Further, we also hypothesized that fMRS measures of GABA, a major inhibitor neurotransmitter, would correlate negatively with task BOLD. In addition to these initial aims, our investigations are also focused on addressing many of the unknown properties of analyzing fMRS data and testing the reliability of this technique in single-subject analyses. For example, it is currently unknown whether subject-specific latencies in detecting neurochemical change occur, and if so, whether or not these latencies are driven by underlying BOLD signal change, anatomical differences, and or if different neurochemicals share latencies profiles. These questions, among many others are of paramount importance for better understanding how to collect, analyze and make use of fMRS as a method for studying brain function in health and in disease.

Fatty acids activate the NLRP3 inflammasome through distinct pathways from canonical NLRP3 triggers

Abstract Authors	Jonathan Liang, Clinton Bradfield, Clare Bryant, Iain Fraser
Graduate Student Name	Jonathan Liang
NIH Institute-Center	NIAID
NIH Research Mentor	Iain Fraser
UK Graduate University	Cambridge University
University Research Supervisor	Clare Bryant
Graduate Department/Program	Veterinary Medicine

The NLRP3 inflammasome is a macromolecular complex that responds to diverse danger signals by inducing secretion of IL-1β and promoting pyroptotic cell death. Inappropriate activity of this complex has been linked to diseases of altered metabolism such as atherosclerosis and type 2 diabetes, and a potential mechanism for that link derives from the recent finding that the NLRP3 inflammasome can be activated by saturated fatty acids (SFAs). However, the mechanisms of SFA activation of the NLRP3 inflammasome remain largely unknown. To study those mechanisms, we have engineered macrophage-like cell lines expressing reporter systems that are compatible with both traditional inflammasome assays and single-cell live imaging analysis. We use these cell systems in combination with inhibitors of specific intracellular processes to compare inflammasome activation by SFAs, other fatty acids, and well-studied NLRP3 triggers such as the pore-forming molecule nigericin. We report that SFAs, though they cause robust gradual ASC puncta formation, induce only modest IL-1β secretion. Furthermore, fatty acid activation of the inflammasome differs from nigericin activation in dependence on reactive oxygen species (ROS) and in sensitivity to reported NLRP3 inhibitors. These findings suggest that fatty acids may engage the NLRP3 inflammasome differently than classic triggers, highlighting the importance of more detailed mechanistic studies of fatty acid-induced inflammasome activation.

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Viral overexpression of NPTX2 to improve inhibitory neuronal functioning and rescue plaque pathology in a mouse model of Alzheimer's disease

Abstract Authors	Connie Mackenzie-Gray Scott; Mandy Lai; Ramesh Chittajallu; Kenneth Pelkey; Chris McBain.
Graduate Student Name	Connie Mackenzie-Gray Scott
NIH Institute-Center	NICHD
NIH Research Mentor	Chris McBain
UK Graduate University	Newcastle University
University Research Supervisor	Andy Trevelyan
Graduate Department/Program	Neuroscience

Alzheimer's disease (AD) is a degenerative, neurological disorder characterised by accumulation of amyloid beta (Ab) plaques resulting in brain circuit dysfunction and memory deficits. The societal burden of AD is worsening due to longer life expectancies and as such, there is a real need to identify novel therapeutics which can restore function to these patients and stop the degenerative processes. Specific brain rhythms, called gamma oscillations, have been shown to be disrupted in mouse models of AD. Gamma oscillations are important for cognitive processes and are generated by the activity of a group of inhibitory interneurons that express the calcium-binding protein parvalbumin (PVINs).

Targeted stimulation of PVINs was shown to restore gamma oscillations and reduce plaque load in a mouse model containing 5 familial AD mutations (5xFAD). Cortical functioning requires that neuronal systems monitor and adjust their activity patterns homeostatically and this is especially important for inhibitory cells such as PVINs. The synaptic protein neuronal pentraxin 2 (NPTX2) is required for the activity-dependent recruitment of PVINs by excitatory pyramidal cells (PCs). Specifically, in response to increased activity it promotes GluA4 subunit expression at PVIN AMPA receptors, enhancing PVIN recruitment. Genetic knockdown of NPTX2 and its receptor significantly reduces expression of GluA4-containing AMPA receptors and consequently impairs PVIN activity, gamma oscillations and cognitive function. Furthermore, NPTX2 levels have been shown to be decreased in the brain and cerebral spinal fluid of AD patients.

Our data from the 5xFAD mice show intracellular Ab deposits by 1 month and Ab plaques by 3 months. Using immunohistochemistry and western blot analysis we wish to determine whether levels of NPTX2 or GluA4 are altered in these mice. Additionally, we will record carbachol-induced gamma oscillations within the CA1 region of the hippocampus, across different time points. This will allow the characterisation of any network deficit developmentally. The next step will be to intervene with viral overexpression of NPTX2 to see if this can rescue any observed deficits associated with PVIN dysfunction and ameliorate the plaque burden seen in these mice. Overall this approach will allow us to define developmentally, PVIN-associated deficits in the 5xFAD model and to test whether targeting the PC-PVIN circuit can function as an alternative therapy in the treatment of AD.

Actions and action values differentially shape recurrent dynamics in the corticostriatal system during learning

Abstract Authors	C. D. Márton, S. R. Schultz, B. B. Averbeck
Graduate Student Name	C. D. Márton
NIH Institute-Center	NIMH
NIH Research Mentor	Bruno B. Averbeck
UK Graduate University	Imperial College London
University Research Supervisor	Simon R. Schultz
Graduate Department/Program	Bioengineering (Computational Neuroscience)

The brain is a non-linear dynamical system and approaching neural processing using methods from dynamical systems offers a way to study how the brain executes complex cognitive tasks (Gallego, J. A. et. al. (2017) Neuron 94:978-984; Mante, V. et. al. (2013) Nature 503:78-84; Chaisangmongkon, W. et. al. (2017) Neuron 3 (6):1504-1517). The prefrontal cortex (PFC) and dorsal striatum (dSTR) enable learning complex behaviours, yet how learning is expressed in top-down signals in these regions remains unclear.

We trained a joint recurrent network model of this brain system on a complex learning task and compared this to recordings made from the PFC and dSTR of two macaque monkeys executing the same task (Seo, M. et. al. (2012) Neuron 74: 947-960). Examining the evolution of task representations with learning across the entire neural population, we found that movement-sequence specific trajectories moved farther apart from each other in latent space with learning in dSTR but not in PFC, both in the neural data and in our model. In our model, this was the result of driving the striatal network to represent action value signals within a reinforcement learning (RL) framework and the prefrontal network to select actions and predict sequence identity based on striatal signals. Further investigating learning dynamics in the striatal network, we uncovered that learning proceeds by shaping gradient manifolds.

The distance between sequence-specific gradient minima increases with learning, and hence, makes it more likely to push the network into the correct sequence-specific trajectory. We also found that task representations became more compact with learning across both brain regions, in the model and recordings. We traced this to changes in the first and second order statistics of the population firing rate with learning. Altogether, we uncovered first evidence of how learning is expressed in top-down signals within the corticostriatal system in the brain, and offered a recurrent neural network model of this system that recapitulated these findings.

Identifying metabolic immunomodulatory mediators of fasting and refeeding using a multi-omics approach

Abstract Authors	Allison Meadows, Dr. Kim Han, Ben McNally, Steven Murfitt, Dr. Antonio Murgia, Dr. Komudi Singh, Dr. James West, Dr. Jing Wu, Dr. Michael Sack, and Prof. Julian Griffin
Student Name	Allison Meadows
NIH Institute-Center	NHLBI
NIH Mentor	Dr. Michael Sack
UK University	Cambridge
University Supervisor	Prof. Julian Griffin
University Department	Biochemistry

Nutrient deprivation through either caloric restriction or fasting has been shown to have a profound impact on reducing risk factors and symptoms of inflammatory disease. Studies have highlighted immune-modulatory and anti-inflammatory effects of nutrient deprivation, suggesting a critical role for metabolic and mitochondrial function on immune activity. To explore the mechanisms underlying these effects, we have carried out a clinical study to assess the immunologic effects of a 24-hour period of fasting followed by a period of re-feeding in normal volunteers. Preliminary results show a fasting-state decrease in activation of the NLRP3 inflammasome and a decrease in activation of CD4+ T cells, and serum collected from these normal volunteers re-capitulates these immune-modulatory effects in vitro.

We hypothesize that circulating metabolites play a pivotal role in regulating nutrient-dependent immune function. To identify these factors or metabolites and to understand the mechanism of these immune-modulatory effects, we have pursued a systems modelling approach that integrates proteomic, transcriptomic, metabolomic, and lipidomic analysis of human serum samples collected through the study. We have so far observed a fasting-state reduction in lysophosphatidylcholines, a class of lipids previously associated with the development of type 2 diabetes, and results to date indicate a potential role for metabolite-sensing G protein-coupled receptors in conferring immune-regulatory effects of these lysophospholipids and other lipid mediators.

Further, we have observed a fasting-associated increase in branched-chain amino acid catabolism, which preliminary studies suggest plays a role in reducing inflammatory signaling in THP1 macrophages. Through functional characterization of identified factors, metabolites, and pathways, we hope to elucidate the mechanisms underpinning fasting-mediated immune modulation and shed new light on the role of nutrient intake on inflammatory pathophysiology and treatment of metabolic disease.

Simultaneous single-cell transcriptome and protein profiling of influenza vaccination responses in humans

Abstract Authors	Matthew P. Mulè, Andrew J. Martins, Jinguo Chen, Foo Cheung, Yuri Kotliarov, Neha Bansal, Arjun Jain, Juan A. Quiel, Brian Sellers, Pamela L. Schwartzberg, CHI Consortium, John S. Tsang
Graduate Student Name	Matthew P Mulè
NIH Institute-Center	NIAID
NIH Research Mentor	John Tsang
UK Graduate University	University of Cambridge
University Research Supervisor	Kenneth Smith
Graduate Department/ Program	Department of Medicine

Molecular and cellular signatures of vaccination responses derived from comprehensive measurements of immune cells in bulk are well described and include elevation of inflammatory/interferon genes 24 hours post vaccination. Immune profiling studies have also consistently shown strong inter-individual heterogeneity in certain vaccine response signatures, including those predictive of antibody response, e.g. the extent of plasmablast cell increases at day 7.

Bulk whole-blood or PBMC transcriptomic measurements are informative yet may miss changes involving low frequency cell populations. In addition, linking single cell protein expression phenotypes from cytometry to bulk transcriptomic signatures remains challenging. The cellular origin of bulk transcriptomic signatures is thus a major unresolved issue in systems vaccinology studies.

Here we adopted a recently developed technology, CITEseq, to simultaneously profile the transcriptome and 83 cell surface proteins in 3000-5000 cells per sample at baseline, and days 1, 7, 100 post vaccination from multiple subjects selected from two NIH human cohorts involving adjuvanted and non-adjuvanted influenza vaccination. By RNA sequencing of single cells (sc-RNAseq) along with DNA barcoded antibodies, CITEseq enabled direct measurement of single cell transcriptomes together with cell surface phenotypes, thus describing the immune response at unprecedented resolution.

We found a high degree of concordance between major immune cell frequencies enumerated by flow cytometry and CITEseq across 20 subjects. Furthermore, Immune cells traditionally classified as a single "type" with cytometry could be further delineated by CITEseq via assessment of transcriptomic state. We also describe an integrated demultiplexing procedure utilizing both sample "hashing" antibodies to resolve timepoint, and genotype to assign cells to the correct donor. We demonstrate the utility of improved experimental and analytical methods for CITEseq such as antibody titration and background antibody count assessment to quantify experimental noise. Finally, we describe the cellular origin of several previously described vaccine response signatures, which are more interpretable than data from scRNAseq alone as they can be defined by surface proteins, either by manual gating or high dimensional graph-based clustering. Our work lays the groundwork for uncovering novel, predictive vaccination response signatures at the single cell, transcriptome-wide level and for defining the cellular origin of bulk immune response signatures from prior immune profiling studies.

This study was supported by the Intramural Research Program of NIAID, NIH.

Interocular contrast differences exploit the relationship between neuronal disparity patterns of activation and perceptual experience.

Abstract Authors	Laura Palmieri, Jenny Read, Bruce G. Cumming
Graduate Student Name	Laura Palmieri
NIH Institute-Center	National Eye Institute, Laboratory of Sensorimotor Research
NIH Research Mentor	Bruce G. Cumming
UK Graduate University	Newcastle University
University Research Supervisor	Jenny Read
Graduate Department/ Program	Wellcome Trust/NIH 4 years PhD fellowship

We studied the effect of interocular contrast differences on the disparity selectivity of neurons recorded extracellularly from V1 of awake fixating monkeys. We presented random dot stereograms at different disparities under four contrast conditions: 1) High contrast (>99%) in both eyes (HH) 2) Low Contrast (20%) in both eyes (LL) 3) High contrast in the dominant eye, low contrast in the non-dominant eye (HL) 4) the reverse (LH).

In the binocular energy model with no contrast gain control, conditions LH and HL reduce the extent of disparity modulation by a factor of 5 (proportional to the contrast). However, the response to binocularly uncorrelated patterns (baseline) is only halved, so the signal to noise ratio becomes poorer. If the monocular gain is increased by a factor of 5 (exactly compensating for the contrast reduction), the disparity tuning curve is identical to that for HH. Changes in gain control after binocular summation affect baseline and modulation equally. We exploit the fact that monocular and binocular gain changes have different effects on baseline and modulation depth to estimate changes in monocular and binocular gains. We estimate the slope and offset the relationship between the HH and LH tuning curves (type II regression) and find the values of monocular and binocular gains in the model that reproduce these.

As already reported in the cat (Truchard et al, 2000), we find that increases in monocular gain are larger than binocular gain (paired t-test, $p < 0.01$, $n = 34$ samples from 17 cells). We also find a correlation between monocular gain changes for the dominant and nondominant eye (Pearson correlation = 0.54, $p < 0.05$). There is considerable heterogeneity in the strength of monocular contrast gain control between neurons. The correlation of gain modulation strength between the two eyes identifies a new property that appears to be matched across eyes in binocular neurons.

Although correlated, monocular contrast gain changes were significantly larger in the non-dominant eye that the dominant eye. This result raises that intriguing possibility that ocularity measures reflect not just the relative strength of excitatory drive from the two eyes, but also reflect the strength of contrast normalization driven from each eye, with the 'non-dominant' eye characterized by a stronger contrast normalization.

In human observers, contrast differences (HL or LH) reduce stereoacuity relative to LL. Two features or our data can explain this. First, changes in monocular gain only partially compensate for the contrast changes. Secondly, there is often a significant increase in binocular gain. Therefore, a simple mechanism may explain this paradoxical phenomenon.

Extracellular Streptavidin-Binding Peptide (SBP) enables magnetic bead purification of transgenic human iPSCs

Abstract Authors	Ryan Prestil, Kenneth Fischbeck, David Rubinsztein, Michael Ward
Graduate Student Name	Ryan Prestil
NIH Institute-Center	NINDS
NIH Research Mentor	Kenneth Fischbeck
UK Graduate University	University of Cambridge
University Research Supervisor	David Rubinsztein
Graduate Department/Program	Cambridge Institute for Medical Research

With the development of modern gene editing technologies such as CRISPR-Cas9, insertion of plasmid cassettes into human iPSCs to form transgenic cell lines has become a commonplace practice. However, the efficiency of correct genomic integration remains low, resulting in a major bottleneck after transfection that requires a lengthy purification process. Antibiotic resistance genes and fluorescent proteins are often used to select for properly edited cells, but inherent disadvantages to these techniques leaves an unmet need for alternative selection agents.

We here expand upon a method originally developed for use in purification of non-adherent primary human lymphocytes, in which a 38-amino acid Streptavidin Binding Peptide (SBP) is fused to the truncated extracellular domain of the Low Affinity Nerve Growth Factor Receptor (LNGFR). When expressed, this construct effectively localizes to the cell surface. Using streptavidin-coated magnetic beads, mixed cell populations can be rapidly enriched to >99% purity; additionally, incubation with fluorescent streptavidin enables temporary live visualization and FACS enrichment without interfering with downstream immunocytochemistry. This technique has been optimized to enrich rare populations (<1%) of human iPSCs following gene insertion, and it is also amenable to select for transient plasmid expression or viral transduction. By modifying the protocol, this process is also effective at negative selection to remove positive cells and enrich for non-expressing cells. These findings present SBP-LNGFR as a novel selection agent for adherent cells which can be easily implemented to enhance gene editing workflows.

Chimpanzee adenovirus-vectored vaccines for Lassa fever

Abstract Authors	Jyothi Purushotham, Robert Fischer, Neeltje Van Doremalen, Teresa Lambe, Sarah Gilbert, Vincent Munster
Graduate Student Name	Jyothi Naidu Purushotham
NIH Institute-Center	Laboratory of Virology, Division of Intramural Research, NIAID
NIH Research Mentor	Dr. Vincent Münster
UK Graduate University	University of Oxford
University Research Supervisor	Professor Sarah Gilbert, Associate Professor Teresa Lambe
Graduate Department/Program	Jenner Institute, Nuffield Department of Clinical Medicine

Lassa virus (LASV), a genetically diverse arenavirus, is the causative agent of an acute hemorrhagic fever endemic to West Africa. The virus circulates in the natal multimammate mouse reservoir and other commensal rodent species. LASV is transmitted to humans through physical contact with infectious rodents or their secretions; human-to-human transmission is documented as well. Over the past three years, the number of cases of Lassa fever has steadily risen in Nigeria, resulting in 20-30% mortality. Given that there are no licensed prophylactics for Lassa fever, the World Health Organization (WHO) has listed LASV as a priority pathogen for vaccine development.

In this study, we evaluated the preclinical immunogenicity and efficacy of a series of Lassa fever vaccine candidates formulated with the chimpanzee adenovirus vector, ChAdOx1. Sequences encoding the glycoprotein precursor and/or nucleoprotein from the Josiah strain LASV were cloned into the ChAdOx1 genome. The immunomodulatory effects of expressing either the post- or pre-fusion form of the LASV glycoprotein trimer were explored. Comparative immunogenicity experiments were carried out in outbred CD1 mice; antigen-specific T-cell and IgG antibody responses were measured by ELISpot assay and ELISA. The detection of vaccine-induced T-cells exhibiting cross-reactivity to the glycoproteins from heterologous LASV strains may imply conferral of immunity against infection by genetically distinct viruses. A vesicular stomatitis virus-based LASV pseudotype has been developed to study vaccine-induced neutralizing antibodies in the sera of immunized mice. The role of non-neutralizing antibodies in the humoral response to vaccination was investigated using an in vitro assay of NK-cell- and macrophage-mediated antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis, respectively.

Thus far, the protective efficacy of a single candidate, ChAdOx1-Lassa-GP (post-fusion) has been assessed in a lethal outbred Hartley strain guinea pig model of Lassa fever. Vaccinated guinea pigs were fully protected from fatal Lassa fever and any clinical symptoms of illness, whereas guinea pigs receiving a control vaccine succumbed to disease. 100% protection was observed after one and two doses of vaccine; GP-specific IgG antibody titers were boosted after a second dose of the vaccine. Sterilizing immunity was not observed.

The role of extra-thymic Aire-expressing cells (eTACs) in Aire-regulated autoimmunity

Abstract Authors	Lindsey B. Rosen, Stefano Maio, Mike Morgan, Steven M. Holland, Georg A. Holländer
Graduate Student Name	Lindsey B. Rosen
NIH Institute-Center	National Institute of Allergy and Infectious Diseases
NIH Research Mentor	Dr. Steven M. Holland
UK Graduate University	University of Oxford
University Research Supervisor	Prof. Georg A. Holländer
Graduate Department/Program	Paediatrics

Establishment and maintenance of T cell tolerance to self-antigens are actively achieved in the thymus and peripheral lymphoid tissues by mechanisms that involve the transcription facilitator autoimmune regulator (Aire). The expression of Aire allows for the promiscuous gene transcription of tissue-restricted antigens, thus contributing to a molecular representation of self-antigens. Aire is expressed both in the thymus and in cells of secondary lymphoid tissue and can typically be detected in a subpopulation of mature medullary thymic epithelial cells (mTECs), activated B cells, and in a dendritic-like population of cells designated eTACs (extra-thymic Aire-expressing cells). Little is known about the precise phenotype and functional importance of these eTACs.

In addition to the previously reported EpCAM+ eTACs, we have identified an EpCAM- eTAC population. eTACs express high levels of costimulatory molecules such as MHC Class II and CD40. These cells appear to be transcriptionally distinct from conventional dendritic cells as RNAseq data demonstrated that eTACs express tissue-redistricted antigens, albeit at lower levels than mTECs. eTACs may therefore contribute to promiscuous gene expression in the periphery.

To elucidate a role of eTACs in peripheral immune tolerance and hence the in prevention of autoimmunity, we have generated several mouse models that differ in their expression of Aire due to a tissue restricted deletion of the gene employing the Cre:loxP DNA recombination system. Mice lacking Aire expression in mature mTECs (designated $Aire^{TEC}$), B cells ($Aire^{B}$), or all hematopoietic cells ($Aire^{CD45}$) will be assessed for immune function and autoimmunity allowing us to draw conclusions about the specific role of eTACs in immune tolerance.

Patrolling ILCs Restrain Mucosal Viral Replication Through Tissue-Wide Delivery of IFN-γ

Abstract Authors	John P. Shannon, Glennys V. Reynoso, Olena Kamenyeva, Daniel T. McManus, Heather D. Hickman
Graduate Student Name	John P. Shannon
NIH Institute-Center	NIAID
NIH Research Mentor	Heather D. Hickman
UK Graduate University	Cambridge University
University Research Supervisor	Geoff L. Smith
Graduate Department/Program	Pathology

Smallpox, caused by infection with variola virus (VARV), led to devastating human pandemics with high mortality until eradication through a global vaccination campaign. Despite eradication, the precise immune mechanisms underlying recovery from infection are incompletely understood. The most common model employed to study smallpox pathogenesis is murine infection with vaccinia virus (VACV), the virus used for smallpox vaccination.

VACV models of smallpox infection commonly employ intranasal or even intravenous routes of infection, with both resulting in widespread viral dissemination. However, VARV infected humans through the oropharyngeal mucosa, after which oral lesions developed and ruptured, spilling copious infectious virus into the saliva (the route of human-to-human spread). Currently, there are no animal models of poxvirus infection of the oral mucosa, and only a handful of reports of infection of the oral cavity with any virus. To understand protection in this critical barrier site, we developed a mouse model of lip (labial mucosal) infection. After labial administration, VACV replicates to high titers and two waves of innate lymphoid cells (ILCs) are recruited in response to infection. Intravital microscopy reveals highly motile ILCs patrol the infected tissue, however, they do not form contacts with infected cells.

Although, we did not directly visualize ILC-mediated killing of virus-infected cells in the tissue, depletion of NK1.1+ cells enhance viral replication. In addition to direct lysis of target cells, ILCs can secrete potent antiviral cytokines including IFN-γ. Using immunohistochemistry, we show that ILCs produce IFN-γ throughout the infected tissue. This widespread disruption of IFN-γ producing ILCs led us to question whether ILCs could endow the epithelium with infection resistance. Antiviral signaling pathway qPCR arrays using isolated RNA from infected tissues reveal a number of upregulated IFN-regulated genes. Furthermore, IFN-γ neutralization greatly enhances viral infection, suggesting that ILCs deliver IFN-γ to the mucosal tissue to restrain viral replication.

Together, these data illustrate the complex nature of immune protection in a critical yet overlooked tissue during viral pathogenesis. Further, with the recent understanding that many viruses including HSV, Ebola, and Zika can be shed for prolonged periods in the saliva, we believe our data provides a new system for the evaluation of these viruses.

Induced Pluripotent Stem Cell Derived Liver Model for the Study of PNPLA3-induced Non-alcoholic Fatty Liver Disease

Abstract Authors	Samantha G. Tilson, Carola M. Morell, An-Sofie Lenaerts, T. Jake Liang, Ludovic Vallier
Graduate Student Name	Samantha G. Tilson
	NIAID
NIH Institute-Center	NIDDK
NIH Research Mentor	T. Jake Liang
UK Graduate University	University of Cambridge
	Pathology
University Research Supervisor	Ludovic Vallier
Graduate Department/Program	Wellcome Trust Sanger Institute and Wellcome Trust-Medical Research Council Stem Cell Institute

Background and Aims: Non-alcoholic fatty liver disease (NAFLD) represents a growing public health burden. NAFLD is defined by accumulation of fat within the liver which ranges in severity from simple steatosis through non-alcoholic steatohepatitis (NASH). Until recently, NAFLD has been considered a largely metabolic disease; however, recent studies have suggested that genetic factors could also majorly influence disease onset and evolution. Accordingly, genome wide association studies (GWAS) have identified the I148M variant in the gene coding for Patatin-like phospholipase domain-containing protein 3 (PNPLA3) which is strongly associated with NAFLD without underlying metabolic disease. However, very little is known about the mechanisms by which it influences disease development and progression. In order to elucidate these mechanisms, we have developed an in vitro model that takes advantage of the unique properties of human induced pluripotent stem cells (hiPSCs) and the CRISPR/CAS9 gene editing technology.

Method: We used CRISPR/CAS9 to generate hiPSC lines with either a complete knock-out of the PNPLA3 gene or with the I148M variant knocked-in. These genetically edited cells were then differentiated into hepatocytes and placed into a 3D system to improve maturity and functionality. The cells were then treated with free fatty acids to induce a NAFLD-like phenotype.

Results: The genetically edited clones showed similar differentiation efficiency toward hepatocytes as wild-type cells thereby suggesting that mutations in the PNPLA3 gene does not affect the differentiation capacity of the hiPSC cells. Following treatment with free fatty acids, the genetically edited cells showed differential lipid accumulation and an altered pattern of response to lipid-induced stress compared to wild type, indicating that PNPLA3 may affect lipid metabolism in our system.

Conclusion: Once fully established and characterized, these hiPSC lines will provide the first opportunity to fully analyze the role of PNPLA3 in the development and progression of NAFLD in vitro.

Profiling the Acquisition and Diversity of Protective Antibodies to Norovirus in a Vietnamese Birth Cohort: Towards Universal Vaccines

Abstract Authors	Jessica van Loben Sels, Dung Tran Thi Ngoc, Luke Meredith, Stanislav Sosnovtsev, Kim Green, Ian Goodfellow, Stephen Baker
Graduate Student Name	Jessica van Loben Sels
NIH Institute-Center	National Institute of Allergy and Infectious Disease
NIH Research Mentor	Dr. Kim Green
UK Graduate University	University of Cambridge
University Research Supervisor	Pr. Ian Goodfellow
Graduate Department/Program	Department of Pathology

Human norovirus (HNV) is the leading cause of viral gastroenteritis, causing an estimated 200,000 deaths in children under 5 years of age annually, especially in low- and middle-income countries. Vaccine design has been confounded by the influenza-like antigenic diversity of HNV (41 genotypes to date) and limited information on adaptive immune responses in naturally-infected populations. Immunization of naive animals with monovalent HNV vaccines has shown limited cross-genotype protection. Protection elicited by monovalent and bivalent vaccine studies in adults has been difficult to interpret due to the unknown histories of HNV exposure that may have predetermined dominant humoral responses. A birth cohort study in Vietnam offered the unique opportunity to study the natural history of HNV infections starting with primary exposure. Stool and sera were collected from 700 children for two years after birth. We hypothesized that early exposure to a diverse population of circulating HNV strains would induce robust, cross-protective humoral immunity, and that the elucidation of antibody repertoires at each sequential time point could inform the design of a universal HNV vaccine.

Humoral protection was measured using a new surrogate neutralization assay that was created and field-tested in this study. Viral attachment is mediated by histo-blood group antigens (HBGAs), carbohydrates displayed on intestinal epithelial cells. Antibodies that block this interaction correlate with protection against HNV infection. Our new assay is a rapid, high-throughput platform for quantifying levels and specificity of HBGA blockade antibodies in serum. We sequenced HNV strains from patient stools and created patient-specific reagents to supplement a screening panel that included local and international strains spanning 12 genotypes. Consistent with studies in naive animals, children exposed to a single HNV genotype developed primarily genotype-specific blockade responses. Children exposed to more than one genotype developed broadly cross-reactive blockade antibodies against the diverse panel of HNV genotypes, including some strains not detected within the Vietnamese community. We defined the specific genotype exposures that stimulated the widest breadth of blockade antibodies and protection from reinfection. This is the first study to explore the role of HNV genotypic diversity in the development of broad protective immunity, which is critical information for vaccine design.

Extreme Outcomes in Multiple Sclerosis: Rare Genetic Variation to the Rescue

Abstract Authors	Alex D. Waldman, Kyle Binder, Gabriele C. De Luca, and Michael J. Lenardo
Graduate Student Name	Alex D. Waldman
NIH Institute-Center	National Institute of Allergy and Infectious Diseases
NIH Research Mentor	Michael J. Lenardo, MD
UK Graduate University	University of Oxford
University Research Supervisor	Gabriele C. De Luca, MD, DPhil
Graduate Department/Program	Department of Clinical Neurosciences

Multiple sclerosis (MS) is the most common neuroinflammatory demyelinating condition affecting the central nervous system (CNS). More than 2 million people worldwide experience significant morbidity and mortality from MS progression, as there is currently only 1 FDA-approved drug for progressive disease. Classically, the etiology of MS has been attributed to an interaction between genetic susceptibilities and environmental exposures ultimately converging on an autoimmune process in which myelin-specific CD4+ cells promote demyelination. Genome-Wide Association (GWA) studies have been utilized to investigate the genetic contribution to MS.

These studies identified HLA-DRB1*1501 as the sole strong susceptibility allele. Subsequent studies highlighted an additional 200 loci with minor to moderate effects on susceptibility. Stemming from these findings, pathologic and clinical corollary studies have implicated HLA-DRB1*1501 status as a mediator of a more severe pathologic outcome. However, additional genetic mediators of progression and extreme outcomes have remained elusive. In contrast to GWA paradigms, next-generation sequencing (NGS) methods provide more precise and valuable information regarding rare deleterious genetic variation that reduces fitness, does not propagate throughout a population, and is therefore missed in GWA studies. Shortages of financial and technical resources have prevented a robust application of NGS methods to MS. Our current research has its basis in rational genetic drug discovery and aims to uncover mechanistic targets for therapeutic development that halt progression.

To accomplish this goal, we will investigate the role of rare pathogenic variants in mediating extreme outcomes by whole exome sequencing (WES) unique MS multiplex families with discordant clinical phenotypes, as defined by disability accumulation metrics (aim 1). We will then validate the functional roles of the identified pathogenic variants mediating progression in familial MS in vitro and in vivo (aim 2). Lastly, we will examine the consequences of newly identified biological pathways on human quantitative neuropathology of sporadic MS (aim 3).

A Novel Human Monoclonal Antibody That Is Highly Effective At Preventing Malaria Infection

Abstract Authors	Lawrence Wang, Rachel Vistein, Barbara Flynn, Lais Periera, Marlon Dillon, Yevel Flores-Garcia, Neville Kisalu, Azza Idris, Jorgen Nelson, Neil King, Stephen Hoffman, Fidel Zavala, Joseph Francica, Robert Seder
Graduate Student Name	Lawrence Wang
NIH Institute-Center	NIH/NIAID/VRC
NIH Research Mentor	Dr. Robert Seder
UK Graduate University	University of Oxford (Jenner Institute)
University Research Supervisor	Dr. Simon Draper
Graduate Dept/Program	D. Phil in Biomedical Sciences

Malaria infection is a major cause of morbidity and mortality worldwide. There is no licensed vaccine against Plasmodium falciparum, the major cause of malaria-associated mortality. Antibodies can prevent malaria infection by targeting sporozoites (SPZ), the infectious form of P. falciparum transmitted by mosquitoes. The major target of anti-SPZ antibodies is the P. falciparum circumsporozoite protein (PfCSP), an abundant surface protein on SPZ that is essential for motility and invasion of hepatocytes. The immunogenic central repeat region of PfCSP has three types of tetrapeptide repeats (1 NPDP, 4 NVDP, and 38 NANP). In lieu of a licensed and/or highly efficacious malaria vaccine, anti-PfCSP mAbs can potentially be used for prophylaxis in itinerant populations (e.g., travelers, military personnel, diplomats) and for elimination campaigns in malaria endemic regions. Several mouse and human monoclonal antibodies (mAbs) have been reported that prevent malaria infection in mice by preferentially binding the immunodominant major repeat sequence, NANP. Recently, several protective mAbs (CIS43, MGG4, MGU12) targeting the unique sequence, NPDP, at the junction of the N-terminus and central repeat region of PfCSP (termed the junctional epitope) have been reported. Here, we report the isolation and characterization of a new panel of 34 anti-PfCSP human mAbs that were isolated from a volunteer immunized with a radiation attenuated P. falciparum sporozoite vaccine (the Sanaria PfSPZ vaccine) and protected from controlled human malaria infection. All 34 mAbs bound recombinant PfCSP and most mAbs (29 of 34, 85%) bound transgenic P. berghei SPZ expressing PfCSP (Pb-PfCSP). Peptide mapping demonstrated that the majority of mAbs (23 of 34, 68%) bound the NANP major repeats and that several mAbs (10 of 34, 29%) bound the junctional epitope. Most newly cloned anti-PfCSP mAbs were IGVH3-33 or IGVH3-30, consistent with previous reports.The abilities of these anti-PfCSP mAbs to prevent malaria infection in vivo were assessed in mice and compared to CIS43, a previously reported protective anti-PfCSP human mAb. One of these mAbs, mAb 9, more potently protected mice from intravenous and mosquito bite SPZ challenge than CIS43 at similar serum levels. mAb 9 is a IGVH3-33 IgG that binds rPfCSP and Pb-PfCSP SPZ comparably to CIS43 and displays relatively high affinity for the junctional epitope but very low affinity for NANP repeats. mAb 9 is a novel, potently protective human mAb that may have potential for further clinical development.

Genome-wide Association Study Identifies Novel Potential Associations with Birth Weight in a Population Indigenous to the Southwestern US

Abstract Authors	Wedekind LE[1,2], Hsueh W-C[2], Olaiya MT[2], Kobes S[2], Knowler WC[2], Baier LJ[2], Mahajan A[3], McCarthy MI[3,4], Hanson RL[2]
Graduate Student Name	Lauren Wedekind
NIH Institute-Center	NIDDK Phoenix Epidemiology & Clinical Research Branch
NIH Research Mentor	Dr Robert Hanson
UK Graduate University	Oxford
University Research Supervisor	Prof Mark McCarthy and Dr Anubha Mahajan
Graduate Department/Program	Nuffield Department of Medicine, DPhil in Biomedical Sciences

BACKGROUND: Epidemiologic studies in many populations have shown that birth weight is associated with risk of type 2 diabetes (T2D) in adulthood. Previous studies involving a population indigenous to the Southwestern U.S. have demonstrated that lower and higher birth weight groups are at higher risk for T2D than those with normal birth weight. We performed a genome-wide association study (GWAS) of birth weight involving 3700 individuals from this community (2037 female; 1663 male).

METHODS: 496,190 single-nucleotide polymorphisms (SNPs) with allele frequency > 1% were directly genotyped using an Axiom array that was designed to capture common variation in this community (Affymetrix; Santa Clara, CA). 4,589,902 genetic variants were imputed using a reference panel specific to this community. Birth weight was ascertained from Arizona state and medical records. T2D status was determined according to American Diabetes Association criteria at a research exam or during clinical care. Individuals were considered to have been exposed to maternal diabetes in utero if their mother had a documented diabetes status before that individual's birth. Individuals were considered not exposed to maternal diabetes in utero if their mother had had a non-diabetic exam at least 1 year after that individual's birth. Birth weight data were rank-based inverse normalized separately by sex and analyzed for genetic associations using a mixed model (SOLAR-Eclipse; Catonsville, MD) accounting for genetic relationships based on genetic markers among all pairs of individuals. The model was adjusted for birth year and the 1st 5 genetic principal components (PCs) in 2 models (models A and B; listed below).

RESULTS
Notably, we also identified the missense variant ABCC8 R1420H (β=0.41 per H allele; p=1.5E-5), whose inactivating mutations cause congenital hyperinsulinemic hyperglycemia of infancy. We previously reported this variant as associated with T2D (OR=2.2, p=3.0E-5).

DISCUSSION: In sum, we identified novel suggestive genetic associations with birth weight, which require confirmation in additional studies. Our logical next steps are to calculate linkage disequilibrium scores to compare with previously published birth weight GWAS results in the Early Growth Genetics (EGG) Consortium, and to generate algorithms to calculate genetic risk scores for birth weight to compare between this population and the EGG Consortium population.

A within-person examination of stress responses, inhibitory control and food intake in women with eating disorders

Abstract Authors	Margaret L. Westwater, Hisham Ziauddeen, Adam Gorka, Christian Grillon, Monique Ernst, Paul C. Fletcher
Graduate Student Name	Maggie Westwater
NIH Institute-Center	NIMH
NIH Research Mentor	Drs Monique Ernst and Christian Grillon
UK Graduate University	Cambridge
University Research Supervisor	Prof Paul Fletcher
Graduate Department/Program	Department of Psychiatry

Stress has profound effects on eating behaviour, which can lead to substantial alterations in food intake and body weight. Among patients with eating disorders, stress has been shown to precede episodic binge eating; however, the physiological mechanisms underpinning this relationship remain poorly understood. Here, we used a within-subjects design to examine the interaction between stress reactivity and motor control in acutely ill women with anorexia nervosa (binge-eating and purging subtype; AN-BP), bulimia nervosa (BN) and healthy controls (HC), relating this to food consumption.

Eighty-five women (AN-BP = 22, BN = 33, HC = 30, matched for age, IQ and, in BN and HC groups, body mass index) completed two functional magnetic resonance imaging (fMRI) scans at Addenbrooke's hospital. Prior to the study session, participants collected waking salivary cortisol samples on two days of their choice while residing at home. Salivary cortisol samples were collected 0, 30, 45 and 60 minutes after waking to capture the cortisol awakening response (CAR), a peripheral indicator of hypothalamic-pituitary-adrenal axis activity. During the study session, volunteers completed the stop-signal anticipation task (SSAT) before and after an induction of either psychological stress or a neutral state. Induction conditions were counterbalanced across participants within each group. The SSAT indexes two types of inhibitory control: proactive inhibition (anticipation of stopping under different degrees of uncertainty) and reactive inhibition (outright stopping). To examine the acute stress response, plasma cortisol was sampled at baseline (i.e., 2 minutes pre-induction) and at five subsequent time points. Finally, participants had a free-choice test meal after each scan.

Functional MRI data were processed using AFNI software and modelled using a region of interest approach in the striatum, pre-supplementary motor area and inferior frontal cortex. Within each subject, we modelled the parametric effect of stop-signal probability (proactive inhibition) and successful stop-signal trials versus failed stop-signal trials (reactive inhibition). Group-level analyses used linear mixed-effects modelling to assess the effect of participant group, induction condition and a group-by-induction interaction on neural correlates of proactive and reaction inhibition. We will use structural equation modelling to test the effects of group, plasma cortisol, CAR and neural responses on eating behaviour. Taken together, these findings will begin to delineate the metabolic and neural mechanisms that subserve the phenomenon of altered eating under stress in women suffering with eating disorders.

Targeting the untranslated regions of SMN2 as a therapeutic strategy for SMA

Abstract Authors	Audrey M. Winkelsas, George G. Harmison, Christopher Grunseich, Carlo Rinaldi, Melissa Bowerman, Matthew J. Wood, Kenneth H. Fischbeck
Graduate Student Name	Audrey M. Winkelsas
NIH Institute-Center	National Institute of Neurological Disorders and Stroke (NINDS)
NIH Research Mentor	Kenneth H. Fischbeck
UK Graduate University	University of Oxford
University Research Supervisor	Matthew J. Wood
Graduate Department/Program	Department of Paediatrics

Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by loss of function mutations in the survival motor neuron 1 gene (SMN1). All patients have at least one copy of a paralog, SMN2, but a C-to-T transition in this gene results in exon 7 skipping in a majority of transcripts. As a result, only 10 to 20 percent of SMN2 transcripts encode the fully functional SMN protein. Nusinersen, an FDA-approved therapeutic for SMA, is an antisense oligonucleotide (ASO) that promotes exon 7 inclusion in the SMN2 transcript. While very successful, this approach has a ceiling effect determined by the abundance of SMN2 transcripts in cells.

Increasing the total pool of SMN2 transcripts, or increasing the translational efficiency of these transcripts, are strategies to overcome the ceiling effect associated with the splice-switching strategy. We sought to determine whether the untranslated regions of SMN2 contain repressive features that limit its expression, targeting of which could increase SMN levels. We found that ASOs targeting the 5' end of SMN2 increase SMN mRNA and protein levels in fibroblasts. We also identified an AU-rich element in the 3'UTR of SMN2 that represses SMN levels. Work is ongoing to understand the mechanism of action of these ASOs, and how these regulatory features may tune SMN expression in different tissues or developmentally. Our results add to the current understanding of SMN regulation and point toward new therapeutic targets for SMA.

Non-apoptotic caspase activity limits tumor growth and immune cell recruitment

Abstract Authors	Derek Xu, Kenneth Yamada, Luis Alberto Baena-Lopez
Graduate Student Name	Derek Xu
NIH Institute-Center	NIDCR
NIH Research Mentor	Kenneth Yamada
UK Graduate University	University of Oxford
University Research Supervisor	Luis Alberto Baena-Lopez
Graduate Department/Program	Sir William Dunn School of Pathology

Over the past decade, caspases have become associated with a plethora of non-apoptotic functions in physiological and pathological situations, including cancer. However, the non-lethal roles of caspases in tumors are still largely unknown. As caspases are also attractive therapeutic targets, filling this basic scientific knowledge gap is key to fully understanding caspase biology and realizing the therapeutic promises linked to these enzymes. To better characterize the caspase involvement in tumorigenesis, we utilize a Drosophila model of hyperplasia induced by concomitantly activating two relevant signaling pathways for human cancer: EGFR and JAK/STAT.

Strikingly, in this experimental paradigm, caspase deficiency promotes tumor growth, not simply due to a lack of apoptosis, but as a result of an increase in tumor cell size. Additionally, caspase activity modifies the tumor microenvironment, limiting the number of immune cells that adhere to tumors. In contrast to previously described tumor models, these effects are independent of the production of reactive oxygen species. JNK signaling is well known to trigger caspase activation, and we also demonstrate that JNK deficiency can rescue many of the caspase-dependent phenotypes and restore cell fate commitment within malignant cells, thus transforming these tumors into benign neoplasias. Altogether, these findings confirm the context-specific nature of caspase activity in tumorigenesis, and indicate the suitability of our tumor model to investigate the interplay of caspases with different signaling pathways and their impact on the tumor microenvironment.

Finally, since the genetic network involving caspases, JNK, and JAK/STAT signaling is similarly required in our tumor setting as during wound healing and regeneration, we propose that these tumors behave as "open wounds," where cells largely fail to acquire defined cell identities, and they proliferate unrestrained.

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Investigating Age-Related Clonal Hematopoiesis in *In Vivo* Rhesus Macaque Model

Abstract Authors	Yifan Zhou,
Graduate Student Name	Yifan Zhou
NIH Institute-Center	NHLBI
NIH Research Mentor	Cynthia Dunbar
UK Graduate University	Cambridge
University Research Supervisor	George Vassiliou
Graduate Department/Program	Sanger's Institute

Clonal hematopoiesis (CH) describes the clonal expansion of blood cells sharing the same somatic mutations. While clonality is a feature of hematological malignancies, recent large deep sequencing studies have revealed that somatic mutations are also prevalent in peripheral blood cells of individuals without overt hematological abnormalities and become increasingly common with age. The phenomenon is thus termed age-related clonal hematopoiesis (ARCH) and represents clonal expansion of hematopoietic stem (HSPC) or progenitor cells carrying somatic mutations resulting in a competitive advantage.

While the risk of eventual progression to hematologic abnormalities or leukemia appears to be higher in individuals with ARCH, the parameters impacting on progression are unclear. In addition, ARCH confers a greater risk for cardiovascular problems and possibly other systemic diseases. However, there is a lack of in vivo model organisms to investigate the mechanisms underlying ARCH development and malignant transformation. Current project aims to address this unmet need via investigation of the rhesus macaque (RM) as a potential model organism for ARCH. First, we are examining the mutation landscape in more than 70 aging RM blood cells for somatic mutations known to be associated with ARCH in humans using. Second, using an established autologous transplant model and DNA barcoding technology, we are tracking clonal expansion in aged RM, and examining the DNA mutational landscape and RNA expression patterns to map the potential pathways underlying clonal expansions in vivo. Findings from this project should inform basic understanding of ARCH and provide predictive models for assessing prognosis in ARCH, as well as platforms for testing of future therapeutic interventions.

Human interleukin-2 receptor β mutations associated with defects in immunity and peripheral tolerance

Abstract Authors	Zinan Zhang[1],[2], Florian Gothe[3],[4], Perrine Pennamen[5], John R. James[1], David McDonald [3], Carlos P. Mata[1], Yorgo Modis[1], Anas M. Alazami[9], Meghan Acres[3], Wolfram Haller[6], Claire Bowen[6], Rainer Doffinger[7], Jan Sinclair[8], Shannon Brothers[8], Yu Zhang[2], Helen Matthews[2], Sophie Naudion[5], Fanny Pelluard[10], Huda Alajlan[9], Yasuhiro Yamazaki[11], Luigi D. Notarangelo[11], James E. D. Thaventhiran[1], Karin R. Engelhardt[3], Hamoud Al-Mousa[12], Sophie Hambleton[3], Caroline Rooryck[5], Kenneth G.C. Smith[1], Michael J. Lenardo[2]
Graduate Student Name	Zinan Zhang
NIH Institute-Center	NIAID
NIH Research Mentor	Dr. Michael Lenardo
UK Graduate University	Cambridge
University Research Supervisor	Professor Ken Smith
Graduate Department/Program	Medicine

Interleukin-2, which conveys essential signals for effective immunity and immunological tolerance, operates through a heterotrimeric receptor. Genetic deficiency of the α or γ chain causes debilitating disease. Here we identify human interleukin-2 receptor (IL-2R) β chain (IL2RB) gene defects as a cause of life-threatening immune dysregulation.

We report three homozygous mutations in the human IL2RB gene of eight individuals from four consanguineous families that cause disease by distinct mechanisms. Nearly all patients presented with autoantibodies, hypergammaglobulinemia, bowel inflammation, dermatological abnormalities, lymphadenopathy, and cytomegalovirus disease. Patient T lymphocytes lacked surface expression of IL-2Rβ and were unable to respond normally to high-dose IL-2 stimulation. By contrast, patient natural killer (NK) cells retained partial IL-2Rβ expression and enhanced cytotoxic function despite abnormal maturation. IL-2Rβ loss of function was recapitulated in a recombinant system, in which mutated alleles encoded proteins with reduced surface expression and IL-2 binding.

Hematopoietic stem cell transplant resulted in resolution of clinical symptoms in one patient; forced expression of wild type IL-2Rβ also increased the IL-2 responsiveness of patient T lymphocytes in vitro. The hypomorphic nature of this disease highlights the significance of variable IL-2Rβ expression between different lymphocyte subsets and maturation stages as a means of modulating immune function. Insights from these patients can inform the development of IL-2-based therapeutics for immunological diseases and cancer.

Student presentations

Wednesday 26 June: 11:30 - 12:45

11:30 - 11:45	William Nathan	Defining the Molecular Mechanisms of Transcription-Associated Interstrand Crosslink Repair
11:45 - 12:00	Nicholas Ader	Molecular and topological reorganization in mitochondrial architecture interplay during Bax-mediated steps of apoptosis
12:00 - 12:15	Zachary Fitzpatrick	Meningeal humoral immunity during homeostasis and neuroinfectious disease
12:15 - 12:30	Samuel Katz	How to TRIAGE your screen hits: What Napoleon's doctor can teach us about big data interpretation and analysis
12:30 - 12:45	Hannah Mason	Microglia Suppress Tau Propagation in Late Stage Tauopathies

Thursday 27 June: 09:30 - 10:45

09:30 - 09:45	Erin Coonahan	An aptamer-based assay for the detection of antimalarial drugs
09:45 - 10:00	Joseph McAbee	An orthotopic xenograft model for studying reirradiation and glioblastoma evolution
10:00 - 10:15	Zinan Zhang	Human interleukin-2 receptor β mutations associated with defects in immunity and peripheral tolerance
10:15 - 10:30	Michael Fernandopulle	RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether
10:30 - 10:45	Kristoffer Haurum Johansen	Identification of Rasa3 as a novel PI3K effector protein in T cell adhesion and homeostasis

Thursday 27 June: 14:00 - 15:00

14:00 - 14:15	Shannon Jane McKie	The characterization of DNA topoisomerase VI from Methanosarcina mazei using single molecule and ensemble methods
14:15 - 14:30	Nicole Mihelson	Visualization of Innate Immune Cell Dynamics During Early Stage Glioblastoma Development
14:30 - 14:45	Gianmarco Raddi	Immunity and Memory Against Malaria: An Atlas of the Mosquito Immune System at Single-Cell Resolution
14:45 - 15:00	Joseph Roney	Impaired lysosome transport to distal axons contributes to autophagic stress in the neurodegenerative lysosomal storage disorder Niemann-Pick Type C