Wild and Comparative Immunology Workshop 2020 – Program final (v5)

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Wild and Comparative Immunology Workshop 2020 – Program final (v5)

MONDAY 7-DEC (GMT = UTC 0); TUESDAY 8-DEC (AEDT = UTC +11)

Andrea Graham

Title: about Where the Wild Things Are: Naturalizing mouse models for immunology

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Alex Bennett

Investigating the intestinal mucus barrier in wild mice

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Abstract:

Wild animals offer a powerful model for studying biological systems under normal environmental conditions. Factors such as seasonal variation, infection history and resource availability are often not considered during studies with laboratory mice, but are pressures faced by wild animals and humans. The aim of my project is to define the ecological factors most strongly associated with variations in the gut barrier, the changes they elicit and how this might affect functional properties of this crucial protective barrier. Two years of trapping on the Isle of May (IoM) has provided a robust data set where histological and molecular characterisation of the mucus barrier can be compared to ecological readouts including microbiome and diet. The acidity of glycans present in the mucus barrier changes predictably with season, gender and location of the animals. Lab studies using wild strains of intestinal parasite T.muris from IoM also reveal altered patterns of glycosylation in infected mice when compared to laboratory strains of parasites. Variations in extrinsic environmental factors has a reliable influence on functional qualities of the mucus barrier in wild animals, the consequences of this may mean altered barrier properties during immune homeostasis or impaired protection in challenging circumstances, including infection or allergy.

Saudamini Venkatesan

Variation in antibody response to vaccine in captive-bred and wild wood mice

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Abstract:
Variation in magnitude of immune response to vaccines can be driven by intrinsic and extrinsic factors such as host sex, nutritional status, pathogen exposure and coinfection. Knowledge of this variation, however, is often derived from laboratory studies on inbred model organisms raised in sterile conditions. To better quantify this variation, we conducted complementary laboratory and field immunisation experiments with captive-bred and wild wood mice (*Apodemus sylvaticus*). Wild and captive mice under differing nutritional regimes were immunised with Diphtheria vaccine and their resulting antibody responses were measured. Overall, captive mice had stronger antibody response that lasted for longer compared to wild mice. Effects of sex, nutrition and body weight on antibody response, although significant, differed under wild and captive conditions. Our work highlights the importance of inclusion of 'dirty' models and field studies to gain a better understanding of the immune response in general, and of vaccine efficacy, in particular.

Gábor Á. Czirják

Sick bats stay home alone: social distancing during the acute phase response in Egyptian fruit bats

Kelsey R. Moreno[1*], Maya Weinberg[1*], Lee Harten[1], Valeria B. Salinas Ramos[2,3], L. Gerardo Herrera M.[3], **Gábor Á. Czirják**[4†], and Yossi Yovel[1,5†]

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Abstract:
While a highly effective strategy, social living carries many risks, such as increased pathogen transmission. How highly social, and especially free-ranging, mammals mitigate this risk is poorly understood. We used lipopolysaccharide (LPS) injection to imitate bacterial infection in both a captive and a free-ranging colony of an extremely social mammal – the Egyptian fruit bat (*Rousettus aegyptiacus*). We monitored physiological and behavioral responses using an arsenal of methods, including on-board GPS and acceleration, video, temperature, and weight measurements, and immune markers. LPS-challenged fruit bats showed fever, increased haptoglobin and lysozyme levels, and classic illness symptoms like weight loss, anorexia, and lethargy. In both colonies, challenged bats actively isolated themselves from the group for two days by leaving the social cluster and avoiding contact. This starkly contrasts the normal, high contact behavior of this species and differs from how social connections to sick individuals are reduced in other vertebrates. Free-ranging bats ceased exiting the roost to forage, extending findings of foraging cessation in laboratory settings to free-ranging animals. Such behaviors likely benefit infected individuals by conserving energy, supporting the immune response, and lowering predation risk while reducing the risk of transmission to group members.

Amanda Patchett

Mesenchymal plasticity of Tasmanian devil facial tumour cells during *in vivo* vaccine trials

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Abstract:
The Tasmanian devil (*Sarcophilus harrisii*) is threatened by the emergence of Devil Facial Tumour Disease (DFTD), comprising two transmissible cancers (DFT1 and DFT2). The development of effective prophylactic vaccines and therapies against DFTD has been restricted by an incomplete understanding of how allogeneic DFT1 and DFT2 cells maintain immune evasion upon activation of tumour-specific immune responses. In this study, we used RNA sequencing to examine tumours from three experimental DFT1 cases. Two devils received a vaccine prior to inoculation with live DFT1 cells, providing an opportunity to explore changes to DFT1 cancers under immune pressure. Analysis of DFT1 in the non-immunised devil revealed a 'myelinating Schwann cell' phenotype, reflecting both natural DFT1 cancers and the DFT1 cell line used for the experimental challenge. Comparatively, immunised devils exhibited a 'dedifferentiated mesenchymal' DFT1 phenotype. A third 'immune-enriched' phenotype, characterised by increased PDL1 and CTLA4 expression, was detected in a DFT1 tumour that arose after immunotherapy. Mesenchymal plasticity and upregulation of immune checkpoint molecules are strategies used by human cancers to evade immune responses. Our results show that similar mechanisms are associated with immune evasion by DFTD cancers, providing novel insights into DFTD survival that will inform the development of effective DFTD vaccines.

Ken Field

Whole-transcriptome gene expression in African fruit bats: life history and parasitic infection

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Abstract:
We wish to better understand the unique physiology of bats and its role in their ability to act as host reservoirs for pathogens that can spill over to humans. We hypothesize that the propensity of bats to serve as reservoir hosts is related to their tendency to favor immune tolerance towards infectious microorganisms, including viruses. To establish the types of immune responses found in bats, under their native conditions, we have studied the whole-transcriptome gene expression patterns of little epauletted fruit bats (*Epomophorus labiatus*) captured in either the rainy or dry seasons in Uganda. This pteropodid bat species, for which we have generated a high quality de novo transcriptome assembly, is closely related to species known to harbor or have exposure to Marburg virus and Ebolaviruses. We have also found a high prevalence of *Hepatocystis* infections in *E. labiatus* and we are examining the transcriptome-wide changes in gene expression that accompany infection with this eukaryotic protozoan parasite. We will present results showing transcriptomic changes in this species related to life history variables, health status, and parasitic infection load. Together, these results provide insight into disease tolerance in bats and will help identify immune pathways that promote tolerance to potential spillover pathogens.

Mauricio Seguel

Anthelmintic treatment dampens immunosenescence in a wild mammal with consequences for survival

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Abstract:
Senescence of the immune system occurs in a number of taxa with consequences for survival, but the underlying drivers of this condition remain obscure. To understand whether common parasite infections contribute to immunosenescence and if antiparasite therapy ameliorates associated survival costs, we examined the impact of long-term anthelmintic treatment on age-related changes in immunity in African buffalo. We show that 6 out of the 11 focal immune traits assessed experienced age-related changes, including increases in inflammatory markers and declines in leukocytes and IFN-γ**.** Interestingly, anthelmintic treatment slowed the rate of age-related changes in all these traits. Mortality was higher among individuals expressing a senescence phenotype consisting of concomitant increases in IFN-γ and declines in lymphocytes. Anthelmintic treatment diminished the expression of this phenotype and buffered its mortality costs. Our findings reveal a role for chronic helminth infection in driving patterns of immune aging and its fitness consequences in wild populations. Intriguingly, these effects can be mitigated using anthelmintic therapy.

TUESDAY 8-DEC (GMT = UTC 0); WEDNESDAY 9-DEC (AEDT = UTC +11)

Simon Babayan

Structure & variability: retracing immune pathways from noisy estimates

1. University of Glasgow

Orsolya Vincze

High innate immune cell concentrations predict elevated cancer risk across mammals and birds

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Abstract:
Although innate immunity plays key roles in the elimination of pathogens and parasites, this defense doesn't come without costs. Species-specific studies have highlighted that chronically activated immune cells exacerbate tissue damage and often exert tumour-promoting effects, by supporting proliferation and survival of neoplastic cells or by modulating neoplastic micro-environments to favour tumour progression. Although the role of innate immunity is increasingly recognized in cancinogenesis, this association has never been tested across species. Here we used data on zoo animals and estimated age-specific cumulative cancer risks in 160 mammalian and 128 avian species. We obtained species-specific white-blood cell profiles from zoo records, representing physiological normal values of healthy individuals. Using phylogenetic regressions, we show that after controlling for body mass, cancer risk in mammals shows strong positive correlation with eosinophil, monocyte and overall white-blood cell counts, but is unrelated to lymphocyte and neutrophil concentrations. Similarly, cancer risk in birds showed significant positive correlation with eosinophils. Our result suggest that elevated cancer risk might be the cost of superior innate immunity across vertebrates. Nonetheless, the co-evolution of immune profile and cancer risk due to other physiological mechanisms can not be ruled out and causalities of the above associations should be further explored.

Rachel Owen

The immunopeptidomes of two transmissible cancers and their host have a common, dominant peptide motif

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Abstract:
The survival of the Tasmanian devil, the largest remaining marsupial carnivore, is threatened by the emergence of two independent lineages of transmissible cancer, Devil Facial Tumour 1 (DFT1) and Devil Facial Tumour 2 (DFT2). To aid the development of a peptide vaccine and to interrogate how histocompatibility barriers can be overcome, we analysed the peptides bound to Major Histocompatibility Complex class I (MHC-I) molecules from Tasmanian devil cells and representative cell lines of each transmissible cancer. Here we show that DFT1+IFNg and DFT2 express a restricted repertoire of MHC-I allotypes compared to fibroblasts, potentially reducing the breadth of peptide presentation. Comparison of the peptidomes from DFT1+IFNg, DFT2 and host fibroblasts demonstrates a dominant motif despite differences in MHC-I allotypes, with preference for a hydrophobic Leucine residue at position 3 and position W of peptides. DFT1 and DFT2 both present peptides derived from neural proteins, which reflects a shared cellular origin that should be exploited for vaccine design. These results suggest that polymorphisms in MHC class I molecules between tumours and host can be 'hidden' by a common peptide motif, providing the potential for permissive passage of infectious cells and demonstrating complexity in mammalian histocompatibility barriers.

Michael Roast

Fitness outcomes in relation to individual variation in constitutive innate immune function

Michael J. Roast[1†], Nataly Hidalgo Aranzamendi[1], Marie Fan[1], Niki Teunissen[1], Matthew D. Hall[1], Anne Peters[1]

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Abstract:
Although crucial for host survival when facing persistent parasite pressure, costly immune functions will inevitably compete for resources with other energetically expensive traits such as reproduction. Optimising, but not necessarily maximising, immune function might therefore provide net benefit to overall host fitness. Evidence for associations between fitness and immune function is relatively rare, limiting our potential to understand ultimate fitness costs of immune investment. Here, we assess how measures of constitutive immune function (haptoglobin, natural antibodies, complement activity) relate to subsequent fitness outcomes (survival, reproductive success, dominance acquisition) in a wild passerine (*Malurus coronatus*). Surprisingly, survival probability was not positively linearly predicted by any immune index. Instead, both low and high values of complement activity (quadratic effect) were associated with higher survival, suggesting that different immune investment strategies might reflect a dynamic disease environment. Positive linear relationships between immune indices and reproductive success suggest that individual heterogeneity overrides potential resource reallocation trade-offs within individuals. Controlling for body condition (size-adjusted body mass) and chronic stress (heterophil-lymphocyte ratio) did not alter our findings in a sample subset with available data. Overall, our results suggest that constitutive immune components have limited net costs for fitness and that variation in immune maintenance relates to individual differences more closely.

Alexander Downie

Epidemiological context and life history interact to influence optimal immune strategies

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Abstract
Both epidemiology and life history are commonly hypothesized to influence organismal immune strategy, and extensive theoretical and empirical investigation has been devoted to these relationships. But the interaction of these two ecological determinants of immune strategy is rarely considered, despite evidence that this interaction might produce effects that differ from those we might derive from just one facet. Here we investigate the interaction of epidemiology and life history as it affects immune strategy through a model of sensitivity and specificity in parasite recognition and response. The model employs a demographic matrix framework that allows for detailed, realistic depiction of life histories and the variation of epidemiological risk across life. We find that variation in reproductive output schedules alone alters optimal immune strategies, but the direction and magnitude of the effect depends on the way that infection risk varies across life. We further find, by exploring life histories from a variety of vertebrates, that the relationship of various life history summary statistics to optimal immune strategy similarly depends on the nature of variation in infection risk. Our results shed light on the complex interactions shaping immune strategy and may prove valuable in interpreting empirical results in ecoimmunology.

Emily Cornelius Ruhs

Impacts of food unpredictability on the balance between energy and immune management in black-capped chickadees

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Keywords: body composition, complement, fat, food unpredictability, haptoglobin, immune function

ABSTRACT

In winter, temperate resident birds are often faced with periodic low food availability. This unpredictability in resources might have a negative impact on immune function, given that immune system support is highly resource dependent. We investigated the balance between energetic and immune management in black-capped chickadees (*Poecile atricapilus*) by manipulating the predictability of resources. The control group received food *ad lib*., while the experimental group received a reduced amount of food on random days and food *ad lib.* on all other days. We measured two key metrics of energetic management (body and fat mass) as well as a suite of immune system components. Compared with controls, experimental birds maintained higher total body and fat mass, had lower acute phase protein concentrations, and had decreased body temperature and lost more body mass during the fever response following injection with lipopolysaccharide. Interestingly, birds in both groups had similar levels of complement lysis, delayed-type hypersensitivity response (phytohemagglutinin), and primary antibody production (keyhole limpet hemocyanin). This experiment demonstrates that black-capped chickadees strategically increase their fat mass in response to decreased food availability and that this might allow the birds to maintain most of the immune system unaltered, except some of the costliest immune components.

James Adelman

Tolerance of infection in a wild songbird: population-level patterns and potential mechanisms

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Abstract:
Tolerance of infection, or minimizing fitness losses without necessarily killing parasites, is now recognized as an important defense among animal hosts. However, we still know little about how tolerance evolves in the wild or the mechanisms that underlie its expression. We addressed this knowledge gap by comparing responses to infection among populations of house finches (*Haemorhous mexicanus*) infected with *Mycoplasma galliespticum* (MG), a bacterial pathogen that emerged in North American finches during the 1990s, causing severe conjunctivitis and significant population declines. We predicted that animals from populations with longer histories of MG endemism would be more likely to express tolerance than those from populations with shorter or no histories of MG endemism. Using experimental infections in wild-caught, immunologically naïve individuals form seven different populations, we found support for this prediction, using the severity of conjunctivitis as a proxy for fitness. Moreover, RNAseq revealed significant differences in patterns of gene expression among a subset of more- and less-tolerant populations. Here we discuss these findings and use our RNAseq data to highlight molecular pathways with the potential to underlie tolerant phenotypes.

Terry Pinfold

RNAScope™ facilitates immunology research without antibodies.

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Abstract:
Studying the Tasmanian devil and most wildlife species brings challenges because of the lack of species-specific reagents. To address this paucity of antibodies new and emerging technologies such as RNAScope™ and RNA flow technologies can be used to target mRNA as markers for immune cells and gene expression at the single-cell level for any species. RNAScope™ is a readily available commercial product. It represents a major advance in RNA ISH approaches which amplifies target-specific signals up to 8000-fold allowing the detection of a single mRNA molecule and suppression of non-specific hybridization. It can target any animal species, plants, bacteria, and viruses including coronaviruses.
Our experiences with RNAScope™ showed it to be a very robust and sensitive way of phenotyping Tasmanian devil immune cells in paraffin-fixed tissue samples. We then adapted the technology to flow cytometry. This allowed us to measure mRNA expression in individual cells and when combined with the limited antibodies available to us we could also detect protein expression in the same cells. This gave us previously unknown insight into the Tasmanian devils' cytotoxic immune cell responses. RNAScope™ is also a valuable tool to validate antibodies, nanobodies, and other probes being developed and used in wildlife immunology.

WEDNESDAY 9-DEC (GMT = UTC 0); THURSDAY 10-DEC (AEDT = UTC +11)

Irene Salinas

What it takes to live on land: the amazing adaptations of the African lungfish immune system

Kathryn Hussey

Expression of non-classical MHC class I in the transmissible cancer Devil Facial Tumour Disease

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Abstract:
Devil Facial Tumour Disease (DFTD) is a transmissible cancer that has circulated in the Tasmanian devil population for >20 years. DFTD has low MHC class I expression due to epigenetic modifications, preventing recognition of mis-matched classical MHC class I molecules by T cells. However, total MHC class I loss should result in natural killer (NK) cell activation due to 'missing self'. We have investigated the expression of a non-classical MHC class I molecule in DFTD as a mechanism for NK cell inhibition. A monoclonal antibody was generated against a devil non-classical MHC class I, Saha-UD. The antibody binds recombinant Saha-UD protein by Western blot, with limited cross-reactivity to classical Saha-UC and non-classical Saha-UK by Western blot and in blocking experiments using recombinant devil MHC class I proteins. 15 DFTD tumours stained by immunohistochemistry showed varied Saha-UD expression, with 8 showing intra-tumour heterogeneity. Immunohistochemical staining for Saha-UD compared with classical MHC class I showed distinct patterns of expression. We have generated an antibody specific for a devil non-classical MHC class I, showing Saha-UD is expressed by DFTD tumours *in vivo*, potentially acting as a mechanism for immune evasion. Further work will investigate whether Saha-UD is an inhibitory ligand for NK receptors.

Michelle Power

Weeds in wildlife microbiomes: ecology and impact of antimicrobial resistant bacteria in wildlife

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Abstract:
Spillover of zoonotic pathogens from wildlife to humans is a primary threat to global health. In contrast, reverse pathogen transmission (zooanthroponosis), whereby pathogens move from humans into wildlife species remains largely unexplored. Globally, increasing urbanisation and habitat loss are driving wildlife species into urban and regional centres, creating a conduit for microbial traffic between humans, domestic animals and wildlife. A primary example zooanthroponosis is the spread of antimicrobial resistant bacteria to wildlife. Conservation management and translocation further establish routes for microbial traffic. For example, captive breeding exposes wildlife to microorganisms not typically encountered in their habitats, and these microorganisms may be spread to conspecifics through translocation. Similarly, wildlife rehabilitation and release also acts as a conduit for exposure to human-associated microbes and spread of these non-endemic microbes within wildlife. This presentation will define the extent if antimicrobial resistance in key Australian wildlife species including flying foxes, Tasmanian devils and possums. The impacts of dissemination of human-associated microbes to wildlife and the implications for wildlife health and disease will be discussed.

Jacques Robert

TLR5 mediated reactivation of quiescent ranavirus FV3 in *Xenopus*

Robert J.[1], De Jesús Andino F. [1], Yen J., Paiola, M.[1]and Samanta M. [2]

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Abstract here (< 200 words)
Ranaviruses such as Frog virus 3 (FV3) are large dsDNA viruses causing emerging infectious diseases leading to extensive morbidity and mortality of amphibians and other ectothermic vertebrates worldwide. Notably, ranaviruses can persist in asymptomatic hosts. We have established *Xenopus laevis* as a reliable experimental organism for studying host interactions with FV3. Despite rapid viral clearance by the *X. laevis* adult robust immune system, FV3 persists quiescent in macrophages of otherwise healthy asymptomatic frogs. We have shown that inflammation induced by intraperitoneal injection of heat-killed *E. coli* (HK-bacteria) can reactivate FV3 in infected asymptomatic frogs, leading to a systemic infection often lethal. Since Toll-like receptors (TLRs) are critical for recognizing microbial molecular patterns, we investigated their possible involvement in inflammation-induced FV3 reactivation. Among the different TLR genes screened, only TLR5 and TLR22, both recognizing bacterial products, showed significant differential expression following FV3 infection and HK-bacteria stimulation. Furthermore, only the TLR5 ligand flagellin induced FV3 reactivation in peritoneal macrophages *in vitro* and *in vivo*. These data indicate that TLR5 signalling pathway can trigger FV3 reactivation, which suggests a role for secondary bacterial infections or microbiome alterations (stress, pollution) in initiating sudden deadly disease outbreaks in amphibian populations with detectable persistent asymptomatic ranavirus.

Kimberly Morrissey

The third domain of T cells: Defining the uniquely mammalian γμ T cell

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An important component of the adaptive immune system are T cells. T cells are divided into two distinct lineages, using either the αβ or γδ T cell receptors (TCRs), and both are found in nearly all jawed vertebrates. Here, we describe a T cell population, γμ T cells, only found in marsupials and monotremes. γμ T cells have a unique TCR structure with a highly diverse, antibody-like V domain that is unusual in being unpaired, much like light-chainless antibodies. An analysis of single cell transcriptomes from unsorted adult opossum thymus, spleen and peripheral blood cells was performed to investigate the presence and phenotype of γμ T cells in this model marsupial. The following conclusions can be drawn: 1) γμ T cells were absent from both adult opossum thymus and peripheral blood; 2) γμ T cells represent 10% of splenocytes and 37% of splenic T cells; 3) 75.5% of splenic γμ T cells are CD8αα^{+} while none express CD4; and 4) γμ T cells have a transcriptome dissimilar from αβ T cells consistent with having a distinct function. Collectively, these results confirm a unique and ancient third lineage of mammalian T cells.

Marcin Wegrecki

Structural characterization of the marsupial γμ T cell receptor

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Abstract:

Most T cells found in jawed vertebrates express functional heterodimeric receptors (TCRs) on their surface formed by either α and β or γ and δ chains. Each chain possesses two domains, an amino-terminal variable domain (V) and a constant domain (C) on the carboxy-terminus (V-C pattern). In most cases, the ability of T cells to recognize diverse antigens relies on the surface (or paratope) located within Vα – Vβ or Vγ – Vδ segments. Recent genomic studies of non-eutherian Mammals identified clusters of genes that resemble the classical TCR loci but surprisingly contain an additional variable segment. The functional product common for Marsupials and Monotremes called 'μ chain' was predicted to contain two variable (Vμ and Vμj) and one constant (Cμ) domains. Single cells analysis of blood and spleen from *Monodelphis domestica* showed that some of the splenic T cells co-express the μ and γ chains suggesting that both polypeptides could form a novel type of T cell receptor, the γμTCR. Using obtained sequences, we generated and structurally characterized two different γμTCRs. Here we present the first complete structure of an atypical TCR. Our findings confirm that the γ and μ chains form a dimer in solution. While the γ chain tightly interacts with the conserved Vμj-Cμ segment of the μ chain, the unpaired N-terminal Vμ domain localizes atop the dimer via a flexible linker. The Vμ resembles VH domains and its CDR3μ loop represents the only highly variable motif within the whole assembly, thus pointing towards its role in antigen recognition.

Jordan Sampson

Olfactory receptors on lymphocytes in a marsupial

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Abstract:

Olfactory receptors (OR) mediate the sense of smell in the olfactory bulb. Recently ORs have been shown to be expressed on non-olfactory cells including immune cells. During an analysis of single cell transcriptomes of opossum splenocytes, we found that ORs were expressed on a subset of T cells, the γμT cells, which are only found in marsupials and monotremes. The OR on γμT cells are members of an OR family that is also uniquely expanded in the genomes of marsupials and monotremes. The majority of ORs on γμT cells (~61%) had homology with human OR14C36 but were not well annotated. To identify specifically which OR14s are present on γμT cells, we annotated this family in the opossum genome. In the opossum, OR14C has 21 members but only four (OR14C2, C3, C5, and C9) are found on γμT cells. Opossum OR14Cs are broadly expressed and found in brain, diaphragm, kidney, ovary, stomach, teste, and thymus. One OR14 subfamily, OR14I, was found to have conserved synteny between the opossum and Tasmanian devil. OR14I has limited expression in opossum tissues but broad expression in devil tissues. These studies provide the basis for future determination of the function of ORs on non-olfactory tissues.

Brian Dolan

Immunogenetic variation at MHC class I loci reflects natural and anthropogenic fragmentation

Brian Dolan[1]

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Abstract:
Desert bighorn sheep (*Ovis canadensis nelsoni*) are a charismatic ungulate native to western North America that live in mountainous regions. In the desert of the southwest United States, populations of bighorn sheep can be isolated by both natural and anthropogenic barriers. Isolation limits gene flow, exacerbates loss of genetic diversity to drift, and thus could affect immune responses to infectious agents. We sought to develop a relatively rapid method for determining MHC class I gene diversity in individual sheep. Both DNA and RNA were isolated from leukocytes collected from 154 adult bighorn sheep from across different mountain ranges in Southern California. Previously identified primers from domestic sheep were used to amplify exons 2 and 3 from OMHC I genes while appending adapters for PacBio circular consensus sequencing. PCR amplicons from individual animals were then subjected to a second round of PCR to append indexes sequences to allow assigning of individual sequences to unique animals. We successfully identified over 40 unique MHC class I sequences expressed by bighorn sheep. We found clear patterns of MHC class I genotypes differentiated by population, which suggests that both natural and anthropogenic-induced population fragmentation can limit the diversity of MHC genes within a given population.

Karen Kadamani

Effects of acute hypoxia on the immune response in naked mole-rats

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Abstract:
Microglia are macrophages in the brain and are activated during infection or injury. For hypoxia-intolerant species, low environmental oxygen also activates microglia. Confoundingly, mounting an immune response is energetically expensive but hypoxia compromises aerobic cellular energy production. Hypoxia-tolerant organisms typically minimize energy demand in hypoxia. We hypothesized that hypoxia-tolerant naked mole-rats (NMRs) would not mount an immune response to hypoxia and that their immune system would be inhibited during hypoxia. To test this, we injected NMRs with saline±1.5mg/kg lipopolysaccharide (LPS) and exposed them to 24hrs of either normoxia (21% O_2) or hypoxia (11% O_2). Following treatment, animals were anesthetized, perfusion-fixed, and organs were collected. Surprisingly, histopathology (on spleen, liver, lungs, and kidneys) indicated that LPS and hypoxia each induce an immune response in NMRs. Analysis of microglial morphology in brain supports these results: the LPS and hypoxia groups had fewer secondary branches, but more total microglia compared to the control group. In the hypoxia+LPS group there were no additive effects. Taken together, our results indicate that hypoxia and LPS treatment each induce immune responses in NMRs, likely via the same cellular pathways. These results are unexpected and suggest that NMRs prioritize immune responsiveness over reducing metabolic demand in hypoxia.

THURSDAY 10-DEC (GMT = UTC 0); FRIDAY 11-DEC (AEDT = UTC +11)

Tony Purcell

Strategies to study host-pathogen interactions and antigen presentation in exotic species

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Abstract:
The spillover of animal borne viruses into the human population hardly needs to be highlighted as we see on a daily basis the impact of the bat borne SARS-CoV-2 virus on our everyday lives. Tools to dissect host-pathogen interactions and adaptive immune responses in humans and common model species such as mice are well developed. However, there is an alarming lack of approaches and reagents to study the virus in reservoir species such as bats or to dissect innate and adaptive immune responses in less common but often more informative model species such as ferrets, hamsters and primates. Here I will present mass spectrometry (MS) based approaches that allow in depth characterisation of virus-host interactions and adaptive immune responses in a range of species. The use of redundant search functions and high quality MS data can provide a rapid portal into the emerging pathogen threats well before more traditional reagents such as antibodies are available.

Phil Askenase

Exosomes that are both antigen-specific and carry a selected gene-regulating miRNA act at the immune synapse to induce APC-derived secondary suppressive exosomes

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Abstract
As a model of chronic infection we induced therapeutic exosomes that both specifically targeted a particular antigen on acceptor cells due to antibody light chains adsorbed to the exosome surface, and also target specific gene functions of the acceptor cells due to the exosome's cargo of a selected miRNA. This dual antigen-specific (via the surface-adsorbed antibody light chains) and gene-specific (via the internalized selected miRNA) therapy has applications in the treatment of cancer, autoimmunity, and allergies.
We used high-antigen-dose-tolerized mouse CD8+ T cells to produce suppressive exosomes that when incubated in vitro with monoclonal anti-ovalbumin light chains were adsorbed to the exosome surface membrane. Then, these antibody light-chain-coated exosomes were incubated with a specific selected miRNA-150 for association with the exosomes. The result was first-acting 1o suppressive exosomes coated with monoclonal light-chain antibodies specific for ovalbumin antigen peptides complexed in MHC on the targeted surface of activated APC and altering function by transferring specific-gene-regulating miRNA-150.
Confocal laser antibody ultra-microscopy showed that human ovalbumin-peptide-surface expressing clonal Rajai B cell APCs pulsed with these 1o exosomes, respond to the transferred miRNA-150 that is identical in mice and humans. In turn these APC made 2o suppressive exosomes formed in their late endosomal multivesicular bodies, then released extracellularly to act, with peptide/MHC-specificity, on Jurkat effector T cells at the APC:T cell TCR immune synapse. These 2o-acting exosomes transferred a different miRNA for strong prolonged inhibition of active in vivo delayed-type hypersensitivity of the monoclonal anti-ovalbumin TCR of the OTII system for days, even when the 1o anti-ovalbumin peptide miRNA-150-positive exosomes are administered orally in a physiological dose at the height of the in vivo response.
This approach may well applications beyond these studies in mice; possibly significant therapeutic applications in cancer, autoimmunity, and allergy.

Stefania D'Alessio

Deiminated proteins and extracellular vesicles profiling in reindeer (*Rangifer tarandus*)

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Abstract
Rangifer tarandus (reindeer, caribou) belong to Cervidae, order Artiodactyla, with sedentary and migratory populations with circumpolar distribution in the Arctic, Northern Europe, Siberia and North America. Reindeer have developed various adaptive strategies to extreme environments and are an important wild and domesticated species. Therefore, novel insights into immune-related markers are of considerable interest. Peptidylarginine deiminases (PADs) cause post-translational protein deimination, which affects target protein function and contributes to protein moonlighting in health and disease. Extracellular vesicles (EVs) participate in cellular communication via transfer of cargo material, and their release is partly regulated by PADs. This study assessed deiminated protein and EV profiles in plasma from sixteen healthy wild female reindeers, collected in Iceland during screening for chronic wasting disease. Plasma-EVs profiles showed a poly-dispersed distribution from 40nm to 500nm, and were characterised by transmission electron microscopy. Deiminated proteins isolated from whole plasma and plasma-EVs were identified by proteomic analysis and protein interaction networks assessed by KEGG an GO analysis, revealing pathways for immunity, gene regulation and metabolism, with some differences between whole plasma and EVs. Deiminated proteins and EVs are candidate biomarkers for reindeer health and may provide information on regulation of immune pathways across phylogeny and in zoonotic disease.

Iris Mair

Eosinophils gone wild: identification of an unusual eosinophil phenotype in wild mice

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Beyond their classical involvement in parasitic infections and allergic inflammation, eosinophils are recognised as playing important roles in tissue homeostasis and repair. Whilst major advances in eosinophil biology have been made using the laboratory mouse, *Mus musculus domesticus*, both host-intrinsic and -extrinsic variables play important roles in shaping the immune system and immune cell phenotype. Thus, the clean, controlled conditions of a laboratory mouse limits its use as a model of animals in their natural habitat, including humans. Here, we describe in detail the phenotype of eosinophils in a wild *M. m. domesticus* population. Eosinophils were present at higher proportions in wild spleen, peritoneal exudate cells, and bone marrow compared to in laboratory mice. Further, wild mouse eosinophils expressed the cell surface marker Ly6G at unusually high levels, levels typically associated with neutrophils in laboratory mice. We hypothesised that the high prevalence of endoparasitic infections seen in our wild mouse population might be driving this unusual eosinophil phenotype. Indeed, when laboratory mice were infected with an endoparasite, a proportion of eosinophils became Ly6G positive. However, levels of Ly6G expression remained below the levels observed in wild mice, indicating that additional ecological variables may be influencing eosinophil phenotype and potentially function.

Sigrun Lange

PADs, post-translational deimination and extracellular vesicle signatures in diverse taxa across phylogeny

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Abstract:
Peptidylarginine deiminases (PADs) are phylogenetically conserved calcium-dependent enzymes which cause post-translational deimination, leading to structural and functional changes in target proteins. Deimination causes neo-epitope generation, affects gene regulation and also allows for protein moonlighting in health and disease. PADs are also a phylogenetically conserved regulator of extracellular vesicle (EVs) biogenesis, which is important in cellular communication.
Our lab has opened up a new platform of comparative animal model research to elucidate roles for PADs and to assess EV profiles and deimination signatures in a range of taxa across the phylogenetic tree, from Arthropoda to Mammals. This includes wild species (Antarctic seabirds, cetaceans, pinnipeds), commercially important species (teleost fish, Mollusca) as well as animals with unusual metabolic, immunological (including anti-viral and anti-bacterial) or anti-cancerous traits (alligator, shark, llama, cow, naked molerat, horseshoe crab). Using KEGG and GO analysis for deiminated protein signatures under normal physiological conditions in these taxa we have identified that critical pathways for immunity, metabolism and gene regulation are deiminated, some of which are common across taxa, while others are species-specific. This highlights

deimination as a putative post-translational "master" switch, allowing for protein moonlighting in conserved pathways in health and disease across the phylogeny tree.

Jonathan Fenn

Variation in Microbiome Structure and Diversity in Wild Field Voles and its Association with Gastrointestinal Immunity.

University of Nottingham

Abstract:
The gastrointestinal microbiome can exert a great deal of influence on the metabolism and health of its host, making it a vital element to consider when studying factors affecting phenotypic variation in wild animal populations. Bacterial communities are, however, complex and dynamic, making such research challenging, particularly in wild animals. Rodent lab models have provided an invaluable tool for understanding the role of commensal gut bacteria, and their relationship with the host environment. Wild rodent populations, therefore, provide a valuable opportunity for exploring how variation in microbiome structure and diversity manifests in 'natural' settings – i.e. genetically diverse populations contending with multiple environmental and infectious factors simultaneously, with controlled lab studies as a comparative framework.

The population of field voles (*Microtus agrestis*) in Kielder Forest (Northumberland, UK) is well characterised and has been subject to longitudinal and cross-sectional analysis over many years. Here we describe the microbial communities of these animals from caecum and faecal tissue, explore patterns of variation within these communities, and find associations between gut immunity and microbiome diversity and structure. We finally discuss how these associations relate to relationships described in laboratory models and what they could mean for the life history of the animals involved.

Matthieu Paiola

Role of the MHC class I-like XNC4 in host-pathogen interaction during *Mycobacteria* infection in *Xenopus laevis*

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Abstract:
Tuberculosis and non-tuberculosis *Mycobacteria* are of major human health concern because of the lack of effective treatments. Development of new therapeutic strategies would benefit by better understanding interactions between mycobacteria and the host immune system. MHC-I-like restricted innate-like (i)T cells (iNKT and MAIT cells) are involved in the early immune response against *Mycobacteria* in mammals. Nevertheless, their role remains to be fully characterized. For the first time outside mammals, iT cells have been characterized in the amphibian *Xenopus*. Notably, reverse genetic approaches have revealed that the non-polymorphic MHC-I-like XNC4 plays a critical role in tadpoles' resistance against *Mycobacterium marinum* (*Mm*). Preliminary results indicate that XNC4 binds unusually long peptides (9-14 mers). To characterize XNC4-antigen presenting cells during *mycobacteria* infection, we have produced a specific nanobody from a synthetic yeast library using rXNC4. Following *Mm* inoculation, recruitment of XNC4^{high+} MHC-II^{high+} macrophages together with iVα45 T cell were detected by flow cytometry at the site of infection, both in tadpoles and frogs. Use of fluorescent *Mm* also revealed that fraction of XNC4+ macrophages were infected. RT-qPCR analysis of adult XNC4+ peritoneal leukocytes at 6 dpi indicate M2-like phenotype. Ongoing work aims to determine whether XNC4+ macrophage polarization is T cell dependent.

Tiffany Kosch

Applied immunology: How genomic selection can be applied to increase disease resistance in threatened species

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Abstract:
Genetic manipulation of resistance to infectious diseases is challenging due to the complex genetic architecture of this trait often involving many genes of small effect size. Despite this, the heritability of disease resistance is relatively high indicating that selective breeding approaches such as genomic selection could be used to increase it. Genomic selection has shown great efficacy in the livestock and aquaculture industries by dramatically increasing the rate of genetic improvement and accuracy of selection while lowering inbreeding rates. I will discuss the application of genomic selection to increase disease resistance and how this approach can be applied to improve the success of captive breeding and reintroduction programs for wildlife threatened by disease.

Nicholas Blackburn

Tackling a turtle tumour threat in South Texas

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Abstract:
Sea turtle fibropapillomatosis (FP) is a tumor promoting disease that is one of several threats globally to endangered sea turtle populations. The prevalence of FP is highest in green sea turtle (*Chelonia mydas*) populations, and historically has shown considerable temporal growth. Despite being first identified in Florida over 80 years ago, FP was not detected in turtle populations in Texas until 2010, and has since grown to a prevalence of over 35%. FP tumors can significantly affect the ability of turtles to forage for food and avoid predation and can grow to debilitating sizes. In the current study, based in South Texas, we have applied transcriptome sequencing to FP tumors and healthy control tissue to study the gene expression profiles of FP. By identifying differentially expressed turtle genes in FP, and matching these genes to their closest human ortholog we draw on the wealth of human based knowledge, specifically human cancer, to identify new insights into the biology of sea turtle FP. We show that several genes aberrantly expressed in FP tumors have known tumor promoting biology in humans, including *CTHRC1* and *NLRC5*, and provide support that disruption of the Wnt signaling pathway is a feature of FP. Further, we profiled the expression of current targets of immune checkpoint inhibitors from human oncology in FP tumors and identified potential candidates for future studies.