Evolutionary scales of the immune system: Setting the agenda
Monday, 2:00-2:30

In order to effectively protect against an ever-shifting array of pathogens, the immune system evolves at many different scales simultaneously. This Workshop will explore recent research and open questions at each of these levels, as well as ways in which they can inform each other. The first session will cover "Evolution of Symbiosis," with an emphasis on how microbiota can complement and regulate the host immune response and its development. In the second session, we will examine the "Evolution of immune repertoires," looking at the ways in which expressed B and T cell receptor repertoires are influenced both by germline evolution at the species level and by a specific history of exposures at the individual level. The third session, "Evolution of immunity" will focus on the emergence and molecular basis of immunity, asking age-old questions of whether histocompatibility systems present in the invertebrates are related by descent to the vertebrate immune system. Finally, "Evolution of immune specialization" will explore the development of dedicated gene families and cell types and the role of complexity in the immune system.

Speakers:

Martin Flajnik

Evolution of symbiosis: Microbiota and mucosal immunity
Monday, 2:30-8:30

The presence of symbiotic microbiota presents a challenge for host immunity, which must distinguish these organisms from potential pathogens. In this session, we will consider how this delicate balance has evolved and how it is regulated. In particular, as first suggested by McFall-Ngai, was the domestication of microbiota a driver for the evolution of adaptive immunity, and, if so, what components where critical for this development? Do commensals provide selection pressure for particular germline Ig/TCR specificities, and how do they influence the complexity of expressed repertoires? Finally, what is the specific role played by mucosal antibody isotypes and when did this specialization emerge?

Speakers:

Gérard Eberl – The "non-self" in the development and regulation of immunity and behavior
Katherine Knight – Immune development in response to microbiota
Irene Salinas – New paradigms in the evolution of mucosal immune systems in vertebrates
Oriol Sunyer – Control of microbiota homeostasis and metabolism by cold-blooded secretory immunoglobulins: The key role of fish IgM
Janelle Ayres – Disease tolerance as a defense strategy and its implications for host-microbe co-evolution

Evolution of immune repertoires: How biases in expressed B and T cell repertoire affect immunity
Tuesday, 9:00-2:15

Expressed repertoires of adaptive immune receptors are shaped both by long-term evolution of the germline and by short-term evolution driven by the specific antigen exposures of a single individual. This session will address the interplay between these scales and the resulting impact on repertoire structure and diversity. We will also examine convergent/public antibody repertoires and mechanisms of immune imprinting.

Speakers:

Marcos Vieira – How do germline-encoded specificities for particular antigens affect the way short-term evolution plays out
Ana Teles – MHC and sex bias: Shaping the systemic T cell repertoire in three-spined stickleback fish
Pierre Boudinot – Neutralizing public antibody responses: An ancient form of defense conserved in fish and mammals
Peji Moghimi – Population-wide deep-regression modeling of convergence across antibody repertoires
Lauren McGough – Original antigenic sin: How first infections "set the stage" and drive individual susceptibility patterns with epidemiological consequences
Zach Montague – The lifecycle of a B cell
Artemis Efstratiou – Analysis of TCR diversity in three-spined sticklebacks
Rose Yin – A model for how T cell-mediated autoimmunity can be triggered by persistent viral infections

Evolution of immunity: The emergence and molecular basis of immunity
Tuesday, 2:45-6:00

One of the most fundamental and founding principles of immunology is that of allorecognition. This session will investigate the “self/non-self” framing of allorecognition and compare it to other potential constructions such as danger and environmental sensing. Specifically, when/how did allorecognition emerge, and how does it relate to the evolution of multicellularity, and are the invertebrate histocompatibility systems related in any way to those of vertebrates? How do microbiota help regulate this distinction and become tolerated as “self”? What is the evolutionary relationship between innate and adaptive immunity, e.g., which innate defense molecules/mechanisms were co-opted by the adaptive immune system?

Speakers:

Tony De Tamaso – Evolution of allorecognition genes in colonial and solitary invertebrate chordates
Matt Nicotra – Allorecognition in a cnidarian – what might it mean for the evolution of self/non-self recognition in animals

Fadi Lakkis – Innate Recognition of Allogeneic Non-self

Evolution of immune specialization: The roles of redundancy and complexity in the immune system
Wednesday, 9:00-3:15

Many immune genes are members of large and diverse families, making it difficult to map evolutionary and functional relationships across species. Our final session will explore this question, including whether immune cell types share common ancestors across species or are the results of descent of convergent evolution (or both). We will also discuss whether apparent redundancy in immune gene families is required for robustness or if they have instead been selected for by environmental complexity and/or specific pathogens.

Speakers:

Jonathan Rast – Strategies and problems in interpreting the functions of homologous regulators in the development and response of divergent immune systems

Sebastian Fugmann – How to define a macrophage: The challenges of identifying evolutionary relationships across a range of metazoans

Jacques Robert – Evolution of classical MHC and nonclassical MHC or MHC like genes, which first emerged and when?

Yuko Ota – Comparative genomics of NKp30 and the functional implications of diverse NK receptor families

Kate Buckley – An analysis of the evolution of metazoan gene families that encode pattern recognition receptors

Mike Criscitiello – Evolutionary insights and therapeutic potential of projecting structural domains that "reach" for lymphocyte antigen recognition

Evolutionary scales of the immune system: Rapporteur's report and open questions
Wednesday, 3:30-5:00

What have we learned? Where would we like to see future research efforts directed?

Speakers:

Chaim Schramm

Speaker Abstracts

Gérard Eberl – The "non-self" in the development and regulation of immunity and behavior
The symbiotic microbiota plays a key role in many functions of its host. We have shown that the microbiota colonizing the intestine early in life is involved in the development and function of lymphoid tissues, and then in setting the reactivity of the immune system in the long term. The microbiota also releases a large quantity and diversity of compounds into the circulation, which affect different aspects of the host's physiology. I will present these and other data and discuss whether the self-nonself concept is really useful for immunology.

Katherine Knight – Immune development in response to microbiota
I will discuss experiments with rabbits in which the intestinal microbiota is required for development of adaptive immunity. During normal development, albeit in the absence of intestinal microbiota, a normal systemic B cell repertoire does not develop. Only specific microbes drive the expansion of B cells, and with microbiota present in a normal vivarium, some B cells never seem to expand. I'll discuss what components of the microbiota drive immune development, and how this may be accomplished?

Irene Salinas – New paradigms in the evolution of mucosal immune systems in vertebrates
I will present new paradigms in the evolution of mucosal immune systems in vertebrates. I will specifically address how mucosal immunity has evolved to adapt to environmental change and how microorganisms (microbiota and pathogens) regulate host immunity-environment communication. I will use the African lungfish as an example to showcase how immunity has supported vertebrates to successfully acquire dual modes of living in water and land. I will also use lungfish to illustrate how mucosal inflammation and mucosal regeneration can be done under physiological conditions with the goal to adapt to extreme environments.

Oriol Sunyer – Control of microbiota homeostasis and metabolism by cold-blooded secretory immunoglobulins: The key role of fish IgM
Throughout evolutionary time, specific immunoglobulin (Ig) isotypes have become specialized in the maintenance of microbiota homeostasis at mucosal sites. In mammals and fish, secretory IgA (sIgA) and IgT (sIgT) respectively predominate at mucosal surfaces and play a critical role in microbiome homeostasis. Recent findings have reported that a large proportion of the fish and human gut microbiota are also coated by sIgM. Thus far, the functional significance of sIgM coating of microbiota remains unknown. Our recent data from teleost fish shows that IgM is critical for the control of microbiota and metabolic homeostasis, thus breaking the paradigm that such control is exerted by only one type of secretory immunoglobulin, either sIgA in tetrapods, or sIgT in fish.

Janelle Ayres – Disease tolerance as a defense strategy and its implications for host-microbe co-evolution
No abstract available.

Marcos Vieira – How do germline-encoded specificities for particular antigens affect the way short-term evolution plays out
Antibodies owe their diversity and potency to the long-term evolution of germline immunoglobulin genes and the short-term evolution of competing B cell lineages expressing different combinations of

those genes. Which lineages win out often depends on subsequent somatic mutations that improve antigen binding, yet lineages using specific germline alleles can have higher affinity than others from the start or a higher propensity to adapt. Do those initial advantages strongly determine the outcome of B cell competition and evolution, or are they mostly overcome by somatic hypermutation? Using simulations, we show that selection for receptors with germline-encoded specificity can lead to similar germline allele frequencies between individuals early in the response. As B cell lineages evolve, however, those early advantages are often overcome by lineages using different germline alleles in different individuals, leading to increasingly contingent patterns of germline allele usage over time. We find patterns consistent with those dynamics in the B cell response of mice experimentally infected with influenza virus. Understanding what conditions favor similar versus contingent allele usage may shed light on the long-term evolution of immunoglobulin genes and help efforts to elicit antibodies with specific germline alleles.

Ana Teles – MHC and sex bias: Shaping the systemic T cell repertoire in three-spined stickleback fish
The T cell receptor (TCR) diversity necessary for the recognition of a broad antigen spectrum is determined by the interaction between T cell receptors (TCRs) and cognate ligands presented by major histocompatibility (MHC) molecules during T cell selection in the thymus. Evolution might have favored an optimal diversity in the copy number-variable MHC, defined by a trade-off between the benefits of presenting a larger number of antigens and avoiding the reduction in the diversity of the mature T cell repertoire due to the effects of self-antigen presentation and negative selection. However, our understanding of how the initial T cell repertoire is shaped is still very limited. Three-spined sticklebacks have a completely functional adaptive immune system and exhibit a natural level of diversity at the MHC. The small size of this wild fish allows an easier estimate of the systemic TCR diversity for each individual, ideal in eco-immunological studies. We have developed a cDNA-based 5'RACE protocol with unique molecular identifiers and a stickleback TCRB gene reference library. By characterizing the systemic TCRB repertoire diversity among male and female naive lab bred individuals belonging to different families and harboring copy number-variable MHC genotypes ranging from low to high diversity, we have directly tested the association between gender, MHC, and naive TCR diversity. Our results contribute to the understanding of the T cell selection process and the balance between the recognition of pathogenic vs self antigens during the evolution of the vertebrate adaptive immune system.

Pierre Boudinot – Neutralizing public antibody responses: An ancient form of defense conserved in fish and mammals
Antibody repertoires are generated by genomic rearrangements during B cell differentiation. Although V(D)J rearrangements lead to repertoires which are mostly different between individuals, a substantial fraction of clonotypes are overrepresented and shared between individuals, in fish as in human or in mice. "Public" or "recurrent" clonotypes distinguish responses found in all animals with the same genetic background from the "private" responses that are specific to each individual. The presence of public clonotypes in the B cell responses of different individuals was reported in studies of the anti-phosphorylcholine T15 response in the mouse, which was associated with the protection of mice against Streptococcus pneumoniae. High-throughput sequencing identified such responses against several pathogens, for example influenza virus, dengue, and Haemophilus influenzae. In several cases these public antibodies were neutralizing. Altogether, these observations revealed an unexpected high

prevalence of recurrent clonotypes in B cell repertoires, which suggests a genetic determinism of VDJ rearrangements shared between individuals and expanded during responses to infections. Hence, the generation of Ig rearrangements expressed in public responses against pathogens may play a key role in fighting infections. Mammals and bony fishes diverged more than 350 million years ago, and their contemporary species are evolutionarily distant. In teleost fish, systemic Ab responses to infections are mainly mediated by IgM, whereas IgT is specialized in mucosal immunity. Teleosts express highly diverse Ig repertoires, that comprise a fair fraction of recurrent components, as reported for example from Atlantic salmon. Fish and mammal antibody responses show important differences: in fish, a weak affinity maturation occurs during secondary responses and fish Ig genes are subjected to much lower somatic hypermutation than mammals (at least compared to mouse and human). Thus, V(D)J germline gene sequences potentially contribute to a greater extent to the mature Ig response in fish than they do in mammals. We found public Abs directed against the rhabdovirus viral hemorrhagic septicemia virus in a teleost fish to be neutralizing, the presence of neutralizing Abs being typically associated with host protect ion during rhabdoviral infections. Furthermore, the public anti-VHSV response was not only observed in a clonal isogenic trout line (in which it had been originally described), but also in wild-type individuals with diverse genetic backgrounds. Many years ago it was proposed that Ig genes involved in the mouse public Ab response against phosphorylcholine had been selected for protection against S. pneumoniae: indeed mice in which these VH genes had been disrupted were highly susceptible to the bacterium. However, whether the generation of public rearrangements is positively selected during the evolution of species remains to be demonstrated. Recurrent clonotypes coding neutralizing Abs in humans have also been identified in several models of common and recurrent infections. Hence, they could constitute a target for natural selection. A parallel can be drawn between such a selective process for recurrent Ag receptors during evolution and the importance of invariant T cells in the repertoire of Xenopus tadpoles in which the TCRb repertoire is dominated by a small number of “predetermined” public rearrangements that are critical for resistance to infection by a common frog ranavirus. We propose that protective public responses represent an evolutionarily conserved mechanism of the Ig-based defences against pathogens, and is present in teleost fish, the earliest bony vertebrate group.

Peji Moghimi – <u>Population-wide deep-regression modeling of convergence across antibody repertoires</u>
Despite the enormous size and genomic diversity, antibody repertoires across populations exhibit significant levels of genomic convergence in the form of "public" clonotypes. Though central for understanding the dynamics of naïve or challenged repertoires, studying convergence has gained increasing importance due to the ongoing SARS-CoV-2 global pandemic, whereby the ability to predict public antibodies from sequence data typically acquired from small cohorts could facilitate vaccine design.
So far, only a few studies have addressed this problem by using a machine learning approach; however, only as a binary classification task, whereby public sequences are defined as being shared across repertoires of all individuals. Such an approach ignores the continuous nature of the task and fails to account for the degree by which antibodies are shared. Here, we address this issue by defining the "degree of commonality" (DoC) of public antibodies as a continuous scale between zero and one, which describes how often a clonotype is observed in the population normalised by the frequency at which the clonotype is observed across the individuals who share it. We developed a bespoke pre-trained Transformer-based deep neural network architecture trained as a regression model on the data from the two single deepest naïve-repertoire sequencing studies. To the best of our knowledge, this model

currently predicts the degree of commonality with the highest levels of accuracy and granularity to date, with an overall mean absolute error (MAE) of 0.083 achieved using a rigorous "leak-free" cross-fold validation protocol. Despite the inherent data imbalance across different levels of DoC, this model performs consistently well even for sequences of higher DoC, which become increasingly rarer as the DoC rises. To tackle the data imbalance problem, we developed a bespoke data analytics pipeline, which down-samples data proportionally to an optimised size that does not violate the summary statistics of the population data.

Lauren McGough – Original antigenic sin: How first infections "set the stage" and drive individual susceptibility patterns with epidemiological consequences

The adaptive immune system enables an individual exposed to a pathogen to develop immunological memory that can be recalled upon future exposures to the same pathogen. A host's earliest exposure(s) to a pathogen are thus critical in shaping their subsequent immune responses. In the case of antigenically evolving pathogens like influenza, immunological memory to one strain may be mismatched against future circulating strains. This so-called "original antigenic sin" that biases an individual's immune response toward their earliest exposures can paradoxically prevent them from generating protective immunity to strains encountered later in life. I will discuss the immunological evidence for original antigenic sin, its observed epidemiological consequences, and my mathematical modeling work studying the impact of original antigenic sin on host-pathogen coevolution at the population level.

Zach Montague – The lifecycle of a B cell

B cells are the central actors in the adaptive system and encode highly diverse and mutable pathogen-engaging receptors. They can counter a multitude of pathogens by directly neutralizing invaders or by storing memory to respond to reinfections efficiently. Upon infection, activated B cells seed germinal centers (GCs) where they hypermutate and are selected for enhanced affinity to pathogens. On longer timescales, memory B cells from previous GC reactions can seed new GCs during reinfection and mutate further; however, the extent of their role in response to reinfections is unclear. Because B cells evolve only in GCs, standard dynamical models with continuous accumulation of mutations fail to describe this interrupted evolution. We introduce a stochastic telegraph process to model the B-cell lifecycle by capturing the entry and exit of B cells from GCs and constraining the accumulation of mutations to the GC residents only. We use this model to reconstruct time-resolved evolutionary histories of B cells from a longitudinal dataset of immune repertoires from individuals with HIV and obtain posteriors of the rates across repertoires and patients. Our results elucidate the lifecycle of B cells and clarify the role of memory B cells in secondary responses on a repertoire-wide scale.

Artemis Efstratiou – Analysis of TCR diversity in three-spined sticklebacks

The vertebrate adaptive immune system is based on lymphocyte recognition of pathogen-derived antigens presented by Major Histocompatibility Complex (MHC) molecules. As the engagement of a peptide-MHC complex with a suitable T-cell receptor (TCR) is the critical first step in the initiation of adaptive immune responses, the availability of a diverse T-cell repertoire, constituted by a pool of broad TCR specificities, is crucial. However, surprisingly little is known regarding the degree of natural variation in the inter-individual diversity and dynamics of the T-cell repertoire, especially during infection. Our research thus examines the qualitative and quantitative variation and dynamics of TCRβ repertoires

within and among individuals of the three-spined stickleback, an eco-evolutionary model species. Here we analyze T cell repertoires of lab-bred sticklebacks that were experimentally exposed to the cestode parasite *Schistocephalus solidus*, known to trigger adaptive immunity. Using NGS sequencing and advanced bioinformatics tools, we investigate TCRβ repertoire size and diversity in relation to infection treatment, family background, and individual MHC diversity. Preliminary analyses indicate a substantial variation of TCRβ repertoires among individuals and across infection status, as expected for a species with a natural level of genetic diversity. Interestingly, infected individuals appear to exhibit higher inter-repertoire overlap than control ones. The existence of public, expanded clonotypes shared by all infected individuals further hints at convergent antigen-specific T-cell responses. Yet -surprisingly- most of the top public clonotypes are shared across experimental groups. Lastly, we show significant biases and a family effect on the usage of V-J gene segments.

Rose Yin – A model for how T cell-mediated autoimmunity can be triggered by persistent viral infections
It has long been known that certain persistent infections can trigger the onset of T cell-mediated autoimmune diseases, but the reasons underlying this phenomenon remain unclear. T cell development in the thymus contributes to creating a largely self-tolerant and pathogen-specific mature repertoire. Some autoreactive T cells survive thymic development and circulate in peripheral tissues, but they usually do not result in autoimmunity. Theoretical and experimental studies suggest that activation of autoreactive T cells does not necessarily lead to a full-blown autoimmune response because a threshold number of T cells need to be activated in response to antigen for T cells to proliferate and differentiate into effector cells. Activation of such a "quorum" of T cells is more likely for foreign antigens compared to self-antigens because of the bias against autoreactive T cells conferred during thymic development. We developed a model for thymic development and then challenged the resulting mature T cell repertoire with varying intensities of infection. Persistent or intense infections were modeled by presenting increasing numbers of foreign antigens. Our results describe key parameters and conditions that result in persistent infections triggering T cell-mediated autoimmunity. Specifically, we describe how T cell activation by multiple foreign antigens can result in weakly autoreactive T cells exceeding the quorum threshold and mounting a response to self-antigens. These results highlight the importance of collective effects for T cell-mediated immunity and its aberrant regulation. Implications of our findings for phenomena such as epitope spreading, as well as possible experimental tests, will also be discussed.

Tony De Tamaso – Evolution of allorecognition genes in colonial and solitary invertebrate chordates
The basal chordate, *Botryllus schlosseri*, undergoes a natural transplantation reaction controlled by a single, highly polymorphic locus, called the *fuhc*. Allorecognition occurs at the tips of an extracorporeal vasculature, and two individuals that share one or both *fuhc* alleles are compatible, and the vessels will fuse, forming a parabiosis between the two individuals. In contrast, individuals with no common *fuhc* alleles between them will reject, an inflammatory reaction that results in melanin scar formation at the point of contact, blocking anastomosis. Fusibility is determined by sharing of a self-fuhc allele, reminiscent of the 'missing-self' mode of recognition utilized by vertebrate Natural Killer (NK) cells. The fuhc locus contains 4 polymorphic proteins encoded within a 70 Kb region. Taking a comparative genomics approach we have characterized the locus in multiple species and found that linkage is conserved, and also include framework genes that are similarly linked, even in species without the allorecognition genes. Previous studies have demonstrated that two putative receptors encoded in the fuhc locus, *fester* and *uncle fester*, are involved in this allorecognition response, and that fusion and

rejection is due to integration of signals from these two proteins. We have found that the fester family genes are present exclusively in colonial ascidian species (specifically, the Styelidae family), and have unexpected haplotype variation. We had previously found three haplotypes, but it now appears there are up to 30 different fester family gene loci arranged in multiple haplotypes that show presence/absence polymorphisms, similar to mammalian innate immune receptors (e.g., KIRs, Ly49s, LILRs, etc.) This indicates that immune receptors associated with allorecognition have evolved using similar genomic mechanisms in distant species.

Matt Nicotra – Allorecognition in a cnidarian – what might it mean for the evolution of self/non-self recognition in animals

Colonial marine invertebrates are capable of allorecognition—the ability to discriminate between their own tissues and those of conspecifics. Allorecognition also occurs in mammals in the context of transplantation and pregnancy. Historically, it has been difficult to determine whether vertebrate and invertebrate allorecognition are related because we have limited data on the molecular basis of invertebrate allorecognition. To address this gap, our lab studies the colonial cnidarian, *Hydractinia symbiolongicarpus*. In nature, *Hydractinia* encrust hermit crab shells, where they encounter each other as they grow. Upon contact, colonies either fuse or reject based on whether they have compatible alleles at two allorecognition genes, called *Allorecognition 1* (*Alr1*) and *Allorecognition 2* (*Alr2*). Both encode type I transmembrane proteins capable of trans (cell-to-cell) homophilic binding that is specific in that allelic isoforms only bind each other if they have similar sequences. We have recently discovered that *Alr1* and *Alr2* are part of a family of 41 *Alr* genes, all of which reside a single genomic interval called the Allorecognition Complex (ARC). Using sensitive homology searches and highly accurate structural predictions, we demonstrate that the Alr proteins are members of the immunoglobulin superfamily (IgSF) with V-set and I-set Ig domains unlike any previously identified in animals. Specifically, their primary amino acid sequences lack many of the motifs considered diagnostic for V-set and I-set domains, yet they adopt secondary and tertiary structures nearly identical to canonical Ig domains. Thus, the V-set domain, which played a central role in the evolution of vertebrate adaptive immunity, was present in the last common ancestor of cnidarians and bilaterians. Unexpectedly, several Alr proteins also have immunoreceptor tyrosine-based activation motifs (ITAMs) and immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails, suggesting they could participate in pathways homologous to those that regulate immunity in humans and flies. Additional preliminary data suggests some Alrs may play a role in the formation of cell-cell junctions. We therefore hypothesize that fusion and rejection are regulated by an interplay of ITAM/ITIM-mediated signaling and cell-cell adhesion and are currently working to test this hypothesis in the lab. In my talk, I will summarize these published and unpublished data, and use them as a starting point to speculate on what this might tell us about the evolution of allorecognition systems in animals. I will also highlight research questions raised by these findings and speculate on what this might mean for the evolution of self/non-self recognition in metazoans as a whole.

Fadi Lakkis – Innate Recognition of Allogeneic Non-self

Our group studies the mechanisms by which mammalian (mouse and human) innate immune cells sense allogeneic non-self. We've discovered that myeloid cells (monocytes and macrophages) sense polymorphisms in the signal regulatory protein-alpha (SIRP-a) via the CD47 receptor and non-self MHC class I molecules via paired immunoglobulin-like receptors-A (PIR-A). Such allogeneic encounters cause

the differentiation of monocytes into antigen-presenting dendritic cells, induce allocytotoxic activity in macrophages, and lead to a memory state in monocytes and macrophages specific to the previously encountered allogeneic MHC class I molecule. Although these phenomena have been uncovered in the context of unnatural allogeneic encounters (cell and organ transplantation), their role in natural allogeneic encounters (for example, pregnancy) remains to be studied. They also prompt one to ask important questions about the evolution of PIR-A molecules, which are encoded by genes in the Leukocyte Receptor Complex (LRC), in relation to the evolution of the MHC and TCR genes.

Jonathan Rast – Strategies and problems in interpreting the functions of homologous regulators in the development and response of divergent immune systems

Comparisons between echinoderms and vertebrates, and between jawed and jawless vertebrates provide insight into different levels of deuterostomes immune system divergence. Both pairs of systems diverged in the distant past, but the framework for comparison is much clearer within vertebrates. Cell type similarities are readily identifiable among jawed and jawless vertebrates, despite radical differences in adaptive immune receptors and divergent genomic histories. Cell type comparison between echinoderms and vertebrates (or even protochordates and vertebrates) is much more difficult. Although much attention is paid to unique features of vertebrate immunity and to the gain of genetic complexity from early vertebrate genome duplications, vertebrates have simultaneously lost immune response genes that are in some cases present across invertebrates. Homologous regulatory genes are shared among the development and function of immune cells in echinoderms and vertebrates. These shared features can form a basis for comparison, but to understand their meaning they must be placed in the context a wider regulatory network. I will discuss strategies and problems in interpreting the functions of homologous regulators in the development and response of divergent immune systems.

Sebastian Fugmann – How to define a macrophage: The challenges of identifying evolutionary relationships across a range of metazoans

Phagocytosis of pathogens and the cytotoxic activity of “killer” cells are the two fundamental cellular defense mechanisms. While a large amount of information has been accumulated about the immune cell with such activities in mammals, there is far less known about the identity of such cells across the invertebrate universe. Immune cells in invertebrates are largely classified based on their morphologies and detailed gene expression-based classifications are just beginning to emerge. We started to define and characterize the immune cell types in echinoderms using scRNAseq analyses using the coelomic fluid as our cell sources and identified subgroups of phagocytic cells that appear to mirror the diversity of macrophages in mice. Subsequent experiments using the intestines as a cell source have been initiated and the results will be discussed. Our aim is to use this data (and other publicly available datasets) to map the evolutionary trajectories of macrophages/phagocytes. In this context we will discuss the challenges of identifying evolutionary relationships between the immune cells and lineages across a range of metazoans - or in other words: how to define a macrophage.

Jacques Robert – Evolution of classical MHC and nonclassical MHC or MHC like genes, which first emerged and when?

The growing number of non-classical MHC-I (class Ib) genes and lineages distinct from classical polymorphic MHC-Ia (class Ia) genes in available sequenced genomes should compel us to reevaluate the evolution of MHC-I and adaptive immunity in jawed vertebrates. Indeed, like mammalian

CD1d/iNKT, a large fraction of class Ib genes may drive the development and function of distinct innate-like (*i*)T cell subsets with limited TCR diversity selected to recognize conserved molecular patterns rather than antigenic peptides and under thymic differentiation program different from conventional (*conv*)T cell education. The expansion and diversification of class Ib/iT cell immune surveillance systems during gnathostome evolution beg to question whether the polymorphic class Ia/conv T cell system was a subsequent specialization that occurred after the emergence of class Ibs. To explore this further, the amphibian *Xenopus laevis* with its well characterized immune system, is attractive. Besides one polymorphic class Ia restricting *conv*T cells, *X. laevis* together with other species of the *Xenopus* genus have an expanded lineage of more than 20 class Ib (*mhc1b-uba*) genes with a phylogeny suggesting species-specific subfunctionalization. While as in mammals, *conv*T cells are dominant in adult frogs, tadpoles rely mostly on several prominent distinct iT cell subsets representing over 75% of T cells and interacting with distinct cognate class Ib molecules. Reverse genetics and tetramer technology unveiled the critical role of *i*V6T cells restricted by *mhc1b-uba10* and *i*V45 T cells interacting with *mhc1b-uba4* in resistance against ranavirus and mycobacteria pathogens, respectively. Potential roles of other *mhc1b-uba* genes are postulated based on their tissue expression profiles including *mhc1b-uba14* in intestine and *mhc1b-uba6* lung mucosal immunity. The expansion and specialization of these *mhc1b-uba14* genes is consistent with a scenario where class I b genes represent forerunners in the evolution of adaptive immunity.

Yuko Ota – Comparative genomics of NKp30 and the functional implications of diverse NK receptor families

Natural killer receptors are fast evolving, diverse immunoreceptor families. Generally, specific types, or families, of NK receptors are found in a limited number of species. However, one NK receptor, NKp30, is evolutionarily conserved from sharks to humans. NKp30 has a non-rearranging V-type immunoglobulin superfamily domain, similar in fine structure to antigen receptors. We hypothesize that NKp30 and antigen receptors derived from a common ancestor and are tracing their emergence in evolution through comparative genomics. We will further discuss the functional implications of diverse NK receptor families.

Kate Buckley – An analysis of the evolution of metazoan gene families that encode pattern recognition receptors

One of the hallmarks of successful immune systems is the ability to generate receptors that detect a vast array of ligands. Vertebrates generate diversity within antigen receptors of the adaptive immune systems, through sophisticated mechanisms of somatic diversification. In contrast, a growing body of evidence suggests that invertebrate taxa employ an alternate strategy in which diversity is maintained within the genome in the form of complex gene families that encode immune receptors. I will present an analysis of the evolution of metazoan gene families that encode pattern recognition receptors with emphasis on the invertebrate deuterostomes.

Mike Criscitiello – Evolutionary insights and therapeutic potential of projecting structural domains that "reach" for lymphocyte antigen recognition

B and T lymphocytes of the jawed vertebrate immune system have structurally, genetically, and evolutionarily related receptors for antigen recognition that initiate can immune responses with remarkable specificity and memory. In general, these receptors' antigen binding sites are evolutionarily

conserved, yet a few very different immunoglobulin structures have been characterized from shark, camelids, and cow B cells. Shark also produce a T cell receptor convergent with a recently described structure of an opossum T cell receptor that similarly eschews the vertebrate norm. The emerging trend of the discovery of smaller, projecting structural domains that “reach” for lymphocyte antigen recognition, and these may lend evolutionary insights and therapeutic potential.