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Meeting Report

Fruit flies like a (rotten) banana

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Introduction

Despite, or perhaps because of, the fact that fruit flies spend much of their lives surrounded by microbes, they do not readily succumb to infectious disease. For nearly fifteen years, *Drosophila melanogaster* has been a fruitful model system for studying the molecular genetics of innate immunity (Fig. 1). This year, studies of infection and immunity featured prominently in several sessions during the 49th Annual Drosophila Research Conference, sponsored by the Genetics Society of America and held in San Diego last April. The breadth of the presentations was a testament to progress in the study of immunity in fruit flies, with research presentations considering not only the molecular biology of cellular and humoral immunity, but also the maintenance of natural genetic variation in immune competence, physiological correlates of immunity, and pathogen virulence mechanisms and interactions with host flies. Furthermore, the complete genome sequencing of 12 species of Drosophila last year[1] allowed the presented work to spill outside of melanogaster and include other Drosophila species.

Genetic Variation and Immune System Evolution

With the completion of the draft sequencing of 12 Drosophila species genomes, it has become much easier to study the genetic bases for interspecific differences in immune capabilities across the Drosophila genus. Tim Sackton (Andy Clark's lab, Cornell University) leveraged the sequenced genome of *D. virilis* to study changes in gene expression in this species after bacterial infection via high-throughput short-read cDNA sequencing. Sackton described a number of genes that are induced or repressed, and identified several candidate novel immune effectors in *D. virilis* through computational and gene expression analysis. To characterize divergence in immune gene regulation over a shorter evolutionary time, Erin Hill (also of Andy Clark's lab) reported on the gene expression irregularities of interspecific hybrids between *D. melanogaster* and its sister species *D. simulans*. Immune gene dysregulation in hybrid offspring is expected to highlight epistatic interactions in immune pathways, since mutations that have naturally accumulated in each species since their last common ancestor may cause epistatic incompatibility in hybrids. Hill found that expression irregularities in hybrids tend to accumulate in the most downstream constituents of both the Toll and Imd signaling pathways, e.g., in antimicrobial peptides, suggesting dysregulation is caused by the cumulative effects of mutations in several upstream genes. Hill is now using hemizygous hybrid flies (having null mutant alleles in the *D. melanogaster* chromosome) to pinpoint specific gene interactions that lead to this regulatory breakdown.

It is by now well established that natural *D. melanogaster* populations harbor genetic variation for immune competence,[2] and Kiyoshi Okado (Kanuka Hirotaka's lab, Obihiro University) showed there is also significant variation in immune performance among laboratory strains with "wild-type" immune systems. Canton-S was particularly resistant and a *white* strain was particularly susceptible to infection by *Listeria monocytogenes*. Okado next tested the correlation of bacterial clearance with three different components of the fly immune system: antimicrobial peptide production, phenoloxidase activity and phagocytosis. Compared to a *white* strain, Canton-S had increased peptide production at early time-points post-infection, increased phenoloxidase activity, and increased hemocyte efficiency in phagocytosing fluorescently labeled heat-killed bacteria. Interestingly, however, the rank order in performance among lab stocks varied depending on whether the flies were infected with *L. monocytogenes*, *Salmonella typhimurium* or *Staphylococcus aureus*.

Environmental and Physiological Correlates of Immunity

The immune competence of an individual can be heightened or limited depending on environmental and physiological variables. Several studies this year were devoted to understanding the influence of these factors on host immune success. Kurt McKean (SUNY Albany) reported on the role of sexual conflict in creating tradeoffs for host immune competence. Comparing fly strains artificially selected under conditions of extreme mate competition (120 males raised with 50 females) to control strains (50 males raised with 50 females), McKean found that both males and females from the sexually selected strains had reduced immune competence. This was most likely the result of an evolutionary shift in resource allocation from immune activity to competition for mates in males, with correlated effects in females. A different kind of sexual selection tradeoff was shown by studying the relationship between gene expression in females, and female fecundity and resistance to bacterial infection. McKean found that expression levels of genes showing female biased expression (higher average expression in females than males) tended to correlate with increased female fecundity and decreased disease

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resistance, while male biased genes showed the opposite effect, indicating that in the absence of sexual genetic conflicts females would have higher fecundity and lower immunity. Sarah Short (Brian Lazzaro's lab, Cornell University) also reported on the role that female-male interactions play on host immunity. It was previously shown that components of the male ejaculate induce immune upregulation in females,[3] but that females actually suffer a transient *reduction* in the ability to resist systemic infection after mating.[4] Short demonstrated that induction of the immune system after mating differs between the reproductive tract and the remainder of the fly, and that while mating does decrease a female's resistance to infection, her results showed a trend toward partial rescue by accessory gland proteins passed to the female in seminal fluid.

It is clear that mounting an immune response is energetically demanding, and that immune pathway activation must be linked to some mechanism of releasing energy stores to immune tissues. Insulin signaling has been shown to play an important role in removing nutrients from circulation and increasing energy stores. Ingrid Hansen (Scott Pletcher's lab, Baylor College of Medicine) presented data to characterize the role of dFOXO, a transcription factor that is deactivated by insulin signaling, on susceptibility to bacterial infection in flies. Previous work in the lab had demonstrated that flies with a null mutation in the insulin receptor substrate protein Chico (a condition that attenuates insulin signaling) are more resistant to bacterial infection.[5] In contrast, mutation and overexpression of dFOXO had no phenotypic effect on infections with *Pseudomonas aeruginosa* or *Staphylococcus aureus*. Interestingly, overexpression of dFOXO decreased fly resistance to *Enterococcus faecalis* infection. Overall, her results suggest that the phenotypic effects of *chico* null mutants are independent of dFOXO activation. Tomas Dolezal and Monika Zuberova (University of South Bohemia) reported that adenosine might act as the signal to release energy stores during infection. Previous work has demonstrated that knocking out the adenosine deaminase gene *ADGF-A* leads to a dramatic increase in extracellular adenosine levels and that *ADGF-A* interacts genetically with the Toll pathway.[6] Here, Dolezal showed that *ADGF-A* mutation leads to decreased energy stores (energetic wasting) and early death. This work suggests adenosine may be a signal produced through the Toll pathway in hemocytes sensing an infection, and that adenosine may cause deactivation of insulin signaling in the fat body, resulting in the release of energy stores to mount the immune response.

Many aspects of organismal physiology are under circadian regulation, and it appears likely that immune system regulation in Drosophila is no different. Jung-Eun Lee (Isaac Edery's lab, Rutgers University) characterized Drosophila survival after infection with *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) at different times in their light/dark cycle and in different circadian rhythm mutant strains. Lee found that immune resistance, measured both by bacterial proliferation and fly survival, peaks during the middle of the night. Circadian mutant flies do not show rhythmicity in immune competence and vary in their overall resistance levels. Interestingly, while *period* mutants are more susceptible, other mutants are more resistant. Similarly, Shawn Butcher (Jaga Giebultowicz's lab, Oregon State University) showed circadian periodicity in susceptibility of *D. melanogaster* to insecticides and is testing whether this is due to circadian expression of detoxification genes.

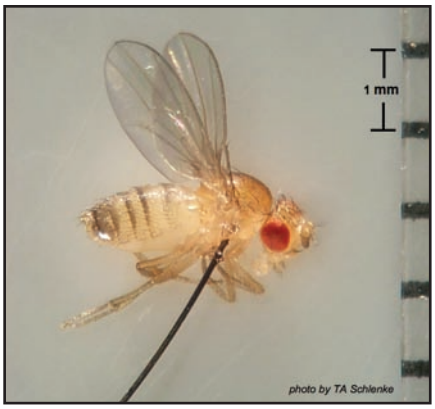

Figure 1. Standard method of infecting Drosophila using a dissecting pin dipped in bacterial culture.

Immune competence also varies with host age. Tashauna Felix (Jeff Leips' lab, University of Maryland Baltimore County) reported on the genetic basis of immunosenescence. *D. melanogaster* strains derived from natural populations show an abundance of genetic variation in age-specific infection susceptibility. Using a representative sample of 12 of these strains, Felix conducted a microarray study of young and old flies, and discovered a module of coordinately regulated genes associated with age-specific defense capability. The functions of most of these genes are unknown, but many were from gene ontology categories previously associated with aging defects, including DNA repair, chromatin remodeling, and microtubule processes, suggesting that immunosenescence may be indicative of a general physiological decline with aging.

One particularly contentious issue in invertebrate immunity is whether invertebrates possess an immune memory akin to that generated by the memory B and T cells of vertebrates. Although immune "memory" has been demonstrated at the physiological level in certain invertebrates infected by certain pathogens, the generality of the phenomena, its mechanistic basis, and its specificity have remained open questions.[7] To distinguish the mechanism of immune memory in vertebrates from that of invertebrates, the phenomenon in invertebrates has been labeled "immunological priming". Following up on the work of Linh Pham, who showed that injecting a priming dose of *Streptococcus pneumoniae* (a Gram-positive bacteria) or *Beauveria bassiana* (a fungus) induces a specific, hemocyte-mediated, long-lasting immune protection against lethal doses of the same pathogen,[8] Junaid Ziauddin (David Schneider's lab, Stanford University) reported data using *Serratia marcescens*, a Gram-negative bacteria. This is interesting because unlike Streptococcus and Beauveria, which primarily induce immune signaling through the Drosophila Toll pathway, immunity against Serratia in flies is assumed to be largely controlled by the Imd pathway. Ziauddin found that priming doses of the bacteria do indeed protect the fly against subsequent lethal doses, and that phagocytosis plays a role in this response, although it is not yet clear whether the priming is specific to *Serratia* infections. One fascinating aspect of the immunological priming literature is that infections of parents can lead to increased immune resistance in their offspring.[9] Jodell Linder (Daniel Promislow's lab, University of Georgia) reported on the potential maternal effects that infected Drosophila females impart to their offspring. Although mothers infected with *Lactococcus lactis*

(Gram-positive) or *Pseudomonas aeruginosa* (Gram-negative) tended to lay fewer eggs, egg size and viability were not affected, and adult offspring were not more resistant to infection than controls. Thus, immune priming in Drosophila does not seem to extend to the offspring of infected mothers.

Another set of presentations dealt with the effects of various toxins on host immunity. Using natural wasp parasitoids of Drosophila, Neil Milan (Todd Schlenke's lab, Emory University) showed that Drosophila that have evolved resistance to toxins associated with natural host plants and fungi (ethanol, octanoic acid and α-amanitin) can use those toxins to help avoid and resist attack by parasitoids. Parasitoids behaviorally avoided fly larvae in toxic substrates, and suffered reduced success developing in larvae grown on food with natural levels of ethanol. This effect is more pronounced with a generalist rather than a specialist parasitoid. In a follow-up experiment using man-made toxins to which *D. melanogaster* has evolved resistance (the insecticides DDT and malathion), the wasp attack rate was not reduced by the presence of the toxins but wasp egg/larval survival in the fly hosts significantly declined, suggesting wasps have not yet evolved the discriminatory ability necessary to avoid flies growing in these toxic substrates or the ability to tolerate the toxic environment inside the fly. Tomasz Krupinski and Iiro Helenius (Greg Beitel's lab, Northwestern University, in collaboration with several others) reported on the detrimental effects of elevated levels of carbon dioxide (hypercapnia) on the Drosophila immune response as a model for similar effects seen in human patients with lung diseases. They found that infected flies and S2 cells in culture induced antimicrobial peptides to significantly lower levels after exposure to bacteria challenge or peptidoglycan in high CO_2 environments than in regular atmosphere. Also, flies in high CO_2 environments showed higher rates of mortality after bacterial infection and lower rates of egg laying and hatching. Krupinski and Helenius further demonstrated that CO_2 suppression does not interfere with NF-κB activation and that the immune effect is independent of pH, nitric oxide level, hypoxia, neuronal CO_2 sensing, and general stress responses, implicating a novel mechanism of CO_2-mediated immune regulatory control.

Bacteria Associated with Drosophila in Nature

Recently, a growing emphasis has been placed on identifying natural bacterial associates of Drosophila. Vanessa Corby-Harris (Therese Markow's lab, University of Arizona) presented her work on the collection of populations of two cactophilic Drosophila species, *D. aldrichi* and *D. arizonae*, and the identification of bacteria associated with these flies using 16S rDNA sequences. She found numerous novel bacterial types, and also found that these cactophilic flies harbored more Gram-positive firmicutes and fewer alpha-proteobacteria than natural populations of *D. melanogaster*.[10]

Other work focused on two bacterial symbionts commonly found in Drosophila and other insects, Spiroplasma species (extracellular, Gram-positive helical bacteria) and *Wolbachia pipientis* (intracellular Gram-negative bacteria). Both are inherited vertically and may cause reproductive abnormalities, such as male-killing and cytoplasmic incompatibility, that increase the spread of the bacteria through fly populations.[11] Tamara Haselkorn (Therese Markow's lab, in collaboration with Nancy Moran, University of Arizona) conducted a multi-locus phylogenetic study of Spiroplasma from nine of the known Drosophila species they infect and found that the Drosophila-associated strains are not monophyletic. Instead, Drosophila have been colonized by Spiroplasmas at least five independent times by four genetically distinct Spiroplasma clades that also infect a variety of other arthropods and plants. Thomas Watts (another member of the collaborative team) studied the natural infection levels of Spiroplasma in host fly species in nature and found that Spiroplasma infection frequencies varied from 5–30% in populations that were infected, with males and females having equal infection rates.

Some of the most exciting work in this area had to do with Wolbachia infection. While conducting a genetic screen to identify *D. melanogaster* loci responsible for resistance to Drosophila C Virus, Luis Teixeira (Michael Ashburner's lab, University of Cambridge) realized that resistance to virus declined dramatically after treating flies with tetracycline, that viral resistance to infection was inherited maternally, and that resistance was correlated with Wolbachia infection. Teixiera confirmed that Wolbachia confers protection to C Virus using several additional *D. melanogaster* strains collected from nature, and then showed that Wolbachia infection also conferred resistance to two other RNA viruses (Nora Virus and Flock House Virus) but not to a DNA virus. This appears to be the first demonstration of a truly beneficial consequence of Wolbachia infection in *D. melanogaster*. Wolbachia are well known to cause cytoplasmic incompatibility in Drosophila, where early lethality of embryos is manifested in crosses between uninfected females and infected males. This gives a reproductive advantage to females who carry the bacteria. It has been unclear as to how Wolbachia cause cytoplasmic incompatibility, however, as the bacteria are not found in male sperm cells. Tim Karr and Ben Heath (University of Bath) reported that Wolbachia phage, recently identified in multiple Wolbachia genome sequences,[12] is assembled into virions and incorporated into developing sperm, which later deliver the virions through the female reproductive track to the egg at fertilization. Karr showed that tetracycline-treated male flies, absent of live Wolbachia, nonetheless still carry virion and still induce the cytoplasmic incompatibility phenotype, suggesting the Wolbachia phage truly act as the causative agent of cytoplasmic incompatibility. Finally, it has been proposed that Wolbachia are refractory to the Drosophila immune system and neither induce nor impair the Toll and Imd pathways in infected insect hosts. Harriet Harris and Lesley Brennan (University of Alberta) undertook a proteomics study using *Aedes albopictus* cell culture to identify host proteins differentially expressed by Wolbachia infection. Several host proteins were upregulated and the cells produced high levels of reactive oxygen species, suggesting that Wolbachia infection is a stress on host cells and they do mount at least a partial immune response.

Pathogen Virulence Mechanisms

Drosophila has been used quite successfully as a model for bacterial and fungal pathogenesis and is poised to become a model for study of viral infections as well. Sara Cherry (University of Pennsylvania) and her lab presented a series of experiments exploring the immune response of Drosophila against viral infection, and the virulence mechanisms viruses use to seize control of host resources. Cherry's lab has established protocols for efficient genomic screening of host genes affecting viral success using RNAi in Drosophila S2 cells.[13] Theresa Moser used this system to study poxvirus infection, by knocking down the 200 kinases and 80 phosphatases in Drosophila. She identified AMP activated kinase (AMPKa), a kinase used in cellular

nutrient signaling, as a necessary host protein for poxvirus growth. AMPKa is phosphorylated and activated following host infection. Claire Marie Filone tested a panel of 1200 pharmacologically active small molecules to identify host determinants necessary for Rift Valley Fever Virus replication in both human and Drosophila cells, and identified calcium signaling inhibitors as conserved inhibitors of viral replication, suggesting that calcium signaling is an important aspect of viral growth. Using the RNAi and small molecule screening protocols, Patrick Rose (in collaboration with Rich Hardy, Indiana University) identified host proteins involved in growth of Sindbis Virus, Sheri Hanna identified host proteins involved in growth of West Nile Virus, and Leah Sabin identified a novel host protein that is anti-viral against a panel of RNA viruses both in cells and whole animals.

Besides free-living viruses, Drosophila hosts must also resist the effects of virus-like particles (VLPs), which parasitic wasps inject into flies along with their eggs to suppress the Drosophila immune response. In parasitic wasps of the genus Leptopilina, the VLPs are produced only in the wasp long gland and have not been shown to carry genetic information into the fly host.[14] Felix Castellanos (Shubha Govind's lab, City University of New York) presented characterization of the morphology and tissue distribution of the VLPs in the long gland of a specialist virulent parasitoid *L. boulardi*-17 (Lb-17). He showed that there are three distinct membrane-bound VLP morphs: spherical particles with spikes, spherical particles without spikes, and filamentous particles. The filamentous particles tend to be found in the long gland itself while the spherical particles with spikes tend to accumulate in the long gland reservoir, which is directly connected to the wasp ovipositor. These structures may represent different VLP developmental stages or may be distinct coexisting microsymbionts.

Two presentations used *D. melanogaster* as a model host to understand how human opportunistic microbial pathogens circumvent innate immunity. Yiorgos Apidianakis (Laurence Rahme's lab, Harvard University, in collaboration with several others) described the function of the gene *Kerv* from the Gram-negative pathogenic bacterium *Pseudomonas aeruginosa*. *Kerv* mutants have attenuated virulence in Drosophila and trigger a more substantial humoral immune response than wild-type strains. Apidianakis showed that the Kerv gene product acts as a positive regulator of components of the Type III secretion apparatus, however, other Type III secretion mutants do not trigger elevated immune reactions. Interestingly, *Kerv* mutants are also defective in lipid metabolism, and Apidianakis proposed that functional Kerv limits production or release of cell wall and membrane components that are recognized by host pattern recognition receptors. Jessica Quintin (Dominique Ferrandon's lab, University of Strasbourg) presented data from infections of flies with *Candida glabrata*, a fungal pathogen responsible for candidiasis. She found that while *C. albicans* is agglutinated by host defense cells, *C. labrata* is not. Furthermore, she found that Toll pathway mutants are more susceptible to *C. glabrata* infection than are wild-type flies, showing that the Toll pathway plays at least some role in immune defense against *C. glabrata*.

Molecular Biology of the Toll and Imd Pathways

Although the characterization of the Toll and Imd humoral immune response pathways in *D. melanogaster* is held up as a major accomplishment in innate immunity research, significant advances continue to be made in their detailed understanding. Lihui Wang (Petros Ligoxygakis's lab, University of Oxford) presented research focused on understanding how three pattern recognition receptors of the Toll pathway (GNBP1, PGRP-SA and PGRP-SD) work jointly to sense peptidoglycans from different pathogens to activate the Toll cascade. Using purified recombinant versions of these proteins, she found that GNBP1 and PGRP-SA bind one another, and that the endomuramidase activity of GNBP1 cleaves peptidoglycan fragments so that they may be more efficiently bound by PGRP-SA. Wang further showed that PGRP-SD is often complexed to both GNBP1 and PGRP-SA, and that PGRP-SD enhances the diversity of peptidoglycans recognized by the GNBP1/PGRP-SA complex. Girish Ratnaparkhi (Albert Courey's lab, UCLA) reported on the function of Dorsal interacting protein 3 (Dip3), which was previously shown to interact with the Toll pathway NFκB transcription factor Dorsal in a yeast-two-hybrid screen.[15] Ratnaparkhi showed that Dip3 synergizes with all three of the Rel NFκB proteins in Drosophila (Dorsal, Dif and Relish). A null mutant in this gene did not have obvious effects on dorsal/ventral patterning of the embryo, as might be expected if the null mutant abolishes embryonic activity of Dorsal, but the Dip3 null mutant flies were significantly more susceptible to Gram-positive and Gram-negative bacterial infections than control flies. Dip3 was found to localize to the promoter region of various antimicrobial peptides, whose expression was reduced in Dip3 mutants, strongly suggesting that Dip3 acts as a transcriptional activator of Rel protein-based innate immune responses.

Three other studies were devoted specifically to the molecular biology of the Imd pathway, which plays an important role in the humoral immune response against Gram-negative bacterial infections. Amy Tang (Mayo Clinic College of Medicine) and colleagues reported that treatment of whole Drosophila, as well as S2 cells, with proteases results in constitutive activation of Imd signaling in a PGRP-LC dependent manner. Because a PGRP-LC construct missing its extracellular domain was found to constitutively activate the Imd pathway, Tang hypothesized that bacteria-mediated damage to the PGRP-LC receptor (and potentially to other immune system components) sets off a hitherto unknown extra layer of host immune regulation to circumvent common virulence strategies of pathogens. Neal Silverman (University of Massachusetts) presented a novel hypothesis about the mechanism of signal transduction in the Imd pathway. He showed that immune stimulation leads to caspase-dependent cleavage of the Imd protein. Furthermore, Silverman showed that the protein dIAP2, which acts downstream of Imd but upstream of the TAK1 and IKK, bound cleaved Imd. Cleavage of Imd was required for the immune-induced ubiquitination of dIAP2. In a separate report, Silverman and colleagues presented a novel finding regarding the regulation of Relish by the Drosophila IKK complex. They found that the IKK complex interacts with the C-terminus of Relish, yet phosphorylates Relish on serine residues in the transcription factor portion of the protein. Signal-dependent phosphorylation of these serines is not required for cleavage, nuclear translocation, or chromosome binding of Relish. Instead, phosphorylation of those residues was shown to control the transcription of Relish-regulated target genes, meaning the IKK complex separately controls Relish cleavage and Relish-regulated transcription.

Cellular Immunity

The cellular half of the immune system in Drosophila is responsible for phagocytosis of microbes and microbial antigens and

encapsulation of larger parasites such as parasitic wasps, and interacts with the humoral signaling pathways in a complex and incompletely understood manner. There are three defined hemocyte lineages in Drosophila: the plasmatocytes are largely responsible for phagocytosis, the crystal cells contain large crystals of the phenoloxidase enzyme that are released into the hemolymph to generate melanin and free radicals, and the lamellocytes are large flattened hemocytes that are induced in third instar larvae to encapsulate parasitic wasp eggs and, potentially, other macroparasites.[16] The work on cellular immunity at the meeting was largely devoted to understanding the genetic and physiological bases of hematopoiesis and hemocyte differentiation, and to understand the molecular mechanisms underlying phagocytosis and encapsulation. Two studies had seemingly conflicting results concerning the role of the JAK-STAT pathway in hemocyte proliferation and lamellocyte differentiation. Rami Makki (Michele Crozatier and Alain Vincent's lab, Toulouse University, in collaboration with several others) showed that JAK-STAT signaling activity is required in the medullary zone of the lymph gland (the larval hematopoietic organ) to maintain a pool of hematopoietic precursors in an undifferentiated state, but that after an immune challenge such as wasp parasitization, the JAK-STAT pathway is repressed, allowing massive differentiation of lamellocytes to occur. Makki found that a novel Domeless-related protein called Latran is required for this repression. Soichi Tanda (Ohio University), however, presented data that seem to show that JAK-STAT activation drives lamellocyte differentiation. Using a mutant from the JAK-STAT pathway gene *hopscotch*, hop^{Tum}, which shows excess hemocyte proliferation, Tanda showed first that the mutant phenotype is temperature dependent, with excess prohemocytes generated at low temperature and excess lamellocyte differentiation seen at high temperature. Addition of a transgenic copy of the hop^{Tum} allele suppressed prohemocyte proliferation and stimulated lamellocyte differentiation, suggesting that the main role of Hopscotch in the JAK-STAT pathway is to induce lamellocyte differentiation. Richard Bou Aoun (Dominique Ferrandon's lab, University of Strasbourg) characterized the role of the thioester containing proteins (TEPs) in *D. melanogaster*. *Tep1* expression was previously shown to be induced by the JAK-STAT pathway in the fat body, while Tep2, 3 and 6 were shown to be important for phagocytosis.[17,18] Bou Aoun found that all five functional TEPs (Tep1, 2, 3, 4 and 6) are expressed by plasmatocytes, and that the expression of *TEPs 1*, *2*, *3* and *4* were induced by bacterial infection. However, constitutive expression of all five *TEP*s also occurred in a variety of larval and adult tissues.

Drosophila larvae will sometimes mount an immune encapsulation response against their own tissues, forming melanotic "pseudotumors". Pseudotumor formation generally occurs in two ways: there can be an overproliferation of hemocytes that begin to encapsulate healthy tissues, or there can be a disruption to the internal basement membrane (such as by an actual tumor) that activates a wild-type encapsulation response.[19] Jose Pastor-Pareja (Tian Xu's lab, Yale University) is using flies double mutant in the *Ras* and *scribble* genes, which exhibit tumor growth similar to human metastatic cancers, to dissect the fly immune response against malignant fly tumors. Specifically, he found that plasmatocytes adhere to the surface of tumors and can restrict tumor growth, and that tumors induce the JAK-STAT pathway and stimulate greater proliferation of hemocytes. Interestingly, artificial disruption of the basement membrane of the fly imaginal discs resulted in hemocyte binding, but did not result in increased hemocyte counts. However, mechanical wounding of imaginal discs caused both hemocyte binding and an increase in the number of hemocytes, suggesting there may be similar mechanisms in the fly responses to tissue damage and tumor growth. Marta Kalamarz and Indira Paddibhatla (Shubha Govind's lab, City University of New York) characterized hematopoietic and immune defects in *lesswright* (*lwr*) mutants. *lwr* is a negative regulator of the Toll/NFκB pathway, and null mutants of this gene result in unrestrained Toll signaling and the production of tumors. Reporting on the size distribution of hematocytic aggregates, they defined "microtumors" as being between 0.5×10^{-3} mm^3–1 mm^3 in diameter. They showed that microtumors are complex and variable, sometimes melanized, and consist largely of hemocytes but occasionally contain fat body tissue. Furthermore, *lwr*/*Dif*/*dorsal* triple mutants do not form microtumors, verifying that the Toll pathway transcription factors Dif and Dorsal play a key role in the process. Oral administration of aspirin relieved the hematopoietic defects found in *lwr* mutants, suggesting that certain aspects of these defects represent symptoms akin to mammalian inflammation. Wei-Ru Li (Henry Sun's lab, National Yang Ming University) and colleagues showed that the Drosophila homolog of *enthoprotein*, encoding a clathrin adaptor protein, is also involved in pseudotumor formation. He found that mis-expression and knockdown/knockout of enthoprotein resulted in melanotic mass buildup, and that this buildup was associated with increased hemocyte counts.

Other Immune Mechanisms

Melanization occurs in Drosophila at the site of a wound and during the melanotic encapsulation of macroparasites and tumors, and is largely controlled by the release and activation of the phenoloxidase enzyme.[20] The melanin is thought to function as a hardening agent. Thomas Hauling (Ulrich Theopold's lab, Stockholm University) and collaborators reported on the different types of elicitors of melanization. Whereas systemic activation of pro-phenoloxidase after infection requires microbial antigens such as peptidoglycan, Hauling found that localized melanization after wounding depends on endogenous signals such as apoptotic cells and their exposed phospholipids. Serpin27A, an inhibitor of the phenoloxidase proteolytic cascade necessary for melanin generation, restricts melanin deposition in the hemolymph to the wound site. Huaping Tang (Carl Hashimoto's lab, Yale University) and collaborators studied the melanization response in the trachea, and identified a novel trachea-specific constitutive inhibitor of the phenoloxidase cascade, Spn77Ba, that restricts tracheal melanization to the site of infection or injury. Active Spn77Ba inhibits a protease cascade involving the proteases MP1 and MP2, a function analogous to the suppression of melanization in the hemolymph by Spn27A. Interestingly, he also found that loss of Spn77Ba function is associated with increased expression of the antimicrobial peptide gene *Drosomycin* in the trachea and fat body, which together with other data suggests that a final product of the melanization reaction acts as an inducer of the systemic humoral immune response.

Both the RNAi and JAK-STAT pathways have been connected to immunity against viral infection in Drosophila.[21,22] Safia Deddouche (Jean-Luc Imler's lab, University of Strasbourg, in collaboration with several others) reported on the gene *Vago*, which

is induced in the fat body after viral infection in a JAK-STAT independent manner, and suppresses proliferation of Drosophila C Virus (DCV). Deddouche and colleagues showed that the Flock House Virus protein B2, which interferes with RNAi through Dicer-2, suppresses induction of *Vago*. She further showed that Dicer-2 is required for induction of *Vago* expression in DCV infected cells, establishing a connection between RNAi and the inducible antiviral response. Sara Cherry (University of Pennsylvania), along with her student Spencer Shelly, presented data from a genomic screen in Drosophila S2 cells of host genes required for inhibiting replication of the generalist viral pathogen Vesicular Stomatitis Virus. The screen identified previously characterized antiviral genes, such as *Dicer-2*. Importantly, they found that knockdown of genes involved in autophagy, such as *Atg8a*, also resulted in increased viral replication both in cells and whole animals. These data suggest hosts use autophagosomes to kill viruses.

Recent work in mammalian systems has identified ATP-dependent potassium channel genes as playing an important homeostatic role during infection.[23] Ioannis Eleftherianos (Jean-Luc Imler's lab, University of Strasbourg) has investigated the role of a Drosophila homolog of the regulatory subunit of the mammalian potassium channel gene *SUR2*, called *dSUR*, which is expressed mainly in the heart. He found that knockdown of *dSUR* in the heart, but not in other tissues, causes susceptibility to infection with the Flock House Virus. In contrast, *dSUR* knocked-down flies are as resistant as wild-type controls to the Drosophila C Virus, and to several bacteria and fungi. Flock House Virus, but not C Virus, is found in the heart after infection where it causes swelling and an increased and irregular beat rate. dSUR associates with the products of the genes *Ir* and *Irk2*, which encode the pore of the potassium channel, and Ir and Irk2 were also found to exhibit protective effects against Flock House Virus infection. Finally, *dSUR* expression declines as flies age, rendering older flies more susceptible to infection.

There has been substantial recent interest in the arthropod gene *Dscam*. Dscam was originally shown to be a neuronal cell membrane receptor involved in cell-cell interactions during neurological wiring,[24] and was secondarily shown to be expressed by Drosophila hemocytes, where it facilitates phagocytosis of bacteria.[25] *Dscam* is massively alternatively spliced, generating >38,000 isoforms in neurological tissue and >18,000 isoforms in immune tissues. Dietmar Schmucker (Harvard Medical School) updated the Drosophila community on the role of *Dscam* in both neuronal development and immunity. He reported that developing multi-dendritic neurons (da neurons) each express more than one *Dscam* isoform, although different da neurons are thought to express different isoform sets. Dscam molecules on neuronal cell membranes undergo homophilic binding, and repulsion occurs between growing dendrites that express the same isoform, which keeps sister dendrites from self-crossing. He also reported on the physical structure of the Dscam protein, showing that the protein folds into a horseshoe shaped structure, one face of which mediates homophilic binding (epitope I) and the other of which exposes a separate variable domain (epitope II). Akhila Parthasarthy (Dietmar Schmucker's lab) reported that the variable epitope II domain contributes to heterophilic binding of largely uncharacterized ligands and may be involved in pattern recognition. Using COS cells expressing particular *Dscam* isoforms, she found that distinct Dscam isoforms mediate specific binding of bacteria, and that the binding specificity resides in epitope II. Thus, more evidence is given that *Dscam* may mediate specificity in the Drosophila immune system through hemocyte pattern recognition.

Concluding Remarks

The 49th Drosophila Research Conference saw an enormous diversity of research presented on the Drosophila immune system and related topics. The breadth of research and increased detail of understanding is impressive if one considers that only fifteen years ago, virtually nothing was known about immunity in Drosophila. The pace with which this field is progressing is inspiring, and we look forward to next year's fly meeting with eager anticipation.

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