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Meeting Report

Fighting Infection Fly-Style

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At the 48th Annual Drosophila Research Conference in Philadelphia, work was presented on multiple aspects of host-microbe interactions. This included identifying new components of known immune signaling pathways—IMD, Toll and JAK/STAT; studies of wounding and cellular responses; exploring pathogenesis with established models (*Pseudomonas, Salmonella, Plasmodia*, parasitoid wasps) and establishing new models; identification of bacterial species that infect Drosophila in the wild; and using the organism to understand the physiology of the immune response.

NEW COMPONENTS OF IMMUNE SIGNALING PATHWAYS

This work was gratifying as it begins to provide validation in vivo for some of the genes identified in cell-based high-throughput RNAi screens. These screens have used the expression of reporter genes for the IMD, Toll, and JAK/STAT signaling pathways. David Kuttenkeuler (Michael Boutros' lab, German Cancer Research Center) reported a novel Toll pathway regulator, DEAF1 (deformed epidermal autoregulatory factor 1). DEAF1 is a DNA binding protein that appears to act downstream of Cactus (IκB homolog) and Dif and Dorsal (NF-κB homologs). In S2 cells, RNAi against Cactus results in increased *Drosomycin* (Toll pathway target gene) expression that can be suppressed by RNAi of DEAF1. Similarly, overexpression of Dif or Dorsal leads to expression of *Drosomycin*, which can be partially suppressed by DEAF1 RNAi. Expression of a transgenic DEAF1 hairpin in vivo resulted in flies that are impaired in their expression of *Drosomycin* in response to *Micrococcus luteus* infection. It will be interesting to determine if DEAF1-RNAi flies are also more susceptible to fungal or Gram-positive bacterial infection. Nadege Pelte (also of the Boutros lab) described the identification of Chifoumi, a putative secreted factor and new negative regulator of the JAK/STAT pathway. Chifoumi is upregulated following a mixed bacterial infection. Overexpression of Chifoumi can block activation of the JAK/STAT pathway in vitro and in vivo. It will be interesting to see the phenotypes of the Chifoumi mutant and how failure to downregulate the JAK/STAT pathway might result in developmental and immune defects. Dominique Ferrandon (CNRS; in collaboration with Neal Silverman, UMass Medical School) described characterization of *TAB2* (TAK1-associated binding protein 2; also known as TAB, CG7417 or *galère*) mutants and their effect in the IMD pathway. Previously, *TAB2* was identified in S2 cells as necessary for induction of the IMD pathway[1,2] *TAB2* was also shown to function with *dTAK1* to activate the JNK signaling and cell death downstream of the TNF homolog *eiger*.[3] The IMD pathway also activates JNK signaling, and dTAK1 is required for both NF-κB and JNK activation in this pathway.[4] In this new presentation, Ferrandon showed that two TAB2 isoforms (long and short) were differentially required for JNK and NF-κB signaling. Molecular and phenotypic analysis of the TAB2 mutant alleles indicated that the long form is required only for the activation of the JNK pathway and is not required for resistance to infection. A TAB2 mutation that affects both long and short forms is susceptible to *E. coli* infection and fails to activate the NF-κB homolog Relish and the expression of antimicrobial peptide genes. Thus, JNK signaling (downstream of dTAK) does not appear to be required for antimicrobial peptide gene expression and may be dispensable for the protection against certain infections. However, reports from others (see below) would argue that the JNK arm of the pathway is essential in mediating protection against other types of infections (*Pseudomonas, Salmonella*). It will be interesting to test whether the TAB2-long mutants are more susceptible in those infection models. Francois Leulier (from Pascal Meier's lab, Institute for Cancer Research) presented new data on *diap2* (Drosophila Inhibitor of apoptosis 2) and its role in the IMD pathway. DIAP2 was previously identified in cell-based RNAi screens as a component of the IMD pathway.[1,5] Characterization of *diap2* mutants in vivo indicated that *diap2* was not necessary to

prevent most apoptosis, but was important for IMD signaling. *diap2* mutants failed to induce IMD target genes and were more susceptible to infection by the Gram-negative bacteria *Erwinia carotovora*.[6,7] While *diap2* transgenes with point mutations in individual BIR domains complemented, RING finger mutant versions of *diap2* failed to rescue the immune deficient phenotype, indicating that the RING ubiquitin-ligase domain is essential for IMD signaling. Similar results have been reported by Bruce Hay's group,[7] who also reported that *diap2* may be important for promoting cleavage of Relish (NF-κB factor in the IMD pathway) and that expression of cell death-inducing genes, *reaper* or *Hid*, leads to a downregulation of DIAP2. This suggests that cell death events may be able to influence the IMD pathway via DIAP2.

HEALING THE WOUND

At the site of injury, epithelial cells activate JNK signaling, presumably to trigger tissue repair, and blood cells secrete factors to mediate clotting and melanization at the wound site.[8,9] Huaping Tang (in Carl Hashimoto's lab, Yale University working with Bruno Lemaitre's group, CNRS) presented an RNAi screen looking at the function of 18 serpin-type inhibitors in vivo. They had previously identified two serine proteases, MP1 and MP2, important for the melanization response,[10] so this RNAi screen could potentially identify the inhibitors for these proteases. He found that many serpins are essential (nine of the 18 are lethal when the hairpin RNAi is expressed via an actin-GAL4). RNAi of 10 of the serpins resulted in constitutive melanization, and RNAi of seven resulted in constitutive *Drosomycin* expression. The phenotypes were not easily categorized, as the various serpins RNAi lines showed some or all of the phenotypes (lethality, melanization, and *Drosomycin* expression). One serpin of interest, Spn77Ba (CG6680), was lethal and showed constitutive melanization in the trachea. The melanization and lethality could be suppressed by a mutation in MP1—suggesting that unregulated activation of MP1 can be lethal and that Spn77Ba might be its inhibitor. Gawa Bidla (in Ulrich Theopold's lab, University of Stockholm) explored the role of melanization in formation of the clot at a wound site.[11] He showed that crystal cells rupture and release prophenoloxidase resulting in melanization of the clot matrix. By screening for failure to either rupture the crystal cells or induce melanization, he found that *eiger* (TNF ligand) and the JNK pathway are necessary and that hemocytes in the clot show hallmarks of apoptosis. Suppression of this apoptosis (by expression of the viral apoptosis inhibitor p35) resulted in reduction of clot melanization. He was able to nicely mimic this with ectopic induction of apoptosis in hemocytes resulting in melanization. This indicates that the JNK pathway and induction of hemocyte apoptosis are important for triggering the melanization of the clot.

CELLULAR IMMUNE RESPONSE, ROLE OF THE TOLL PATHWAY

Nina Matova (in Kathryn Anderson's lab, MSKCC) reported on the roles for Dif and Dorsal in the cellular immune response.[12] The hemolymph of *Dif dorsal* double mutant larvae is filled with microbes and has reduced numbers of hemocytes. These hemocytes were able to engulf bacteria, but were unable to clear them. This overwhelming bacterial infection is the likely cause of the larval lethality, as antibiotic treatment resulted in improved hemocyte morphology and greater numbers of animals surviving to adulthood. Expression of either *dorsal* or *Dif* in the hemocytes rescued all the observed phenotypes. Expression of either *dorsal* or *Dif* in fat body also rescued the numbers of hemocytes, suggesting that a factor from the fat body can influence hemocyte survival. The reduction in hemocyte numbers in the double mutant appears to be due to increased cell death. Expression of *dIap1* (*Drosophila inhibitor of apoptosis 1*) in the hemocytes increased the hemocyte numbers and improved the numbers of double mutant animals surviving to adulthood (from 6.7 to 38.5%). The inability to clear microbes from the hemocytes may cause those cells to die; alternatively or additionally, Dif and Dorsal may be acting to inhibit programmed cell death. Work reported by Jinu Abraham (in Soichi Tanda's lab, Ohio University) also supported the model of Toll pathway components influencing hemocyte numbers and survival. Mutations in *lesswright* (Ubc9 homolog; SUMO conjugating enzyme) result in activation of the Toll pathway.[13] *lesswright* mutants show high numbers of hemocytes and melanotic masses, a phenotype similar to loss of function mutations in *cactus* (IκB homolog). A *lesswright cactus* double mutant resulted in fewer blood cells. This decrease in blood cells is likely due to increased apoptosis, as assayed with acridine orange staining of the cells. It is curious that these two mutations, that both lead to the Toll pathway activation, result in a phenotype similar to the *Dif dorsal* double mutant, which has no Toll signaling. The apoptosis may be occurring via activation of the JNK pathway, as a *lesswright*, JNK dominant negative double 'mutant' displayed increased numbers of hemocytes and decreased apoptosis. Michaela Fenckova (in Tomas Dolezal's lab, University of South Bohemia) presented evidence that increased amounts of adenosine (a nucleotide metabolite) in the hemolymph contribute to the formation of melanotic tumors and lethality in activated Toll pathway mutants.[13] ADGF-A (adenosine deaminase) is an enzyme that degrades adenosine, keeping levels of extracellular adenosine low. The *ADGF-A* mutation is larval lethal, with the mutant larvae showing high levels of adenosine in their hemolymph, disintegrating fat body tissue, and melanotic tumors. Aspects of the *ADGF-A* phenotype are similar to that of *cactus* (or activation of the Toll pathway). They find that overexpression of *ADGF-A* can suppress melanotic tumors and partially rescue *cactus* lethality. This suggests that adenosine signaling is downstream of Toll signaling and that adenosine may serve as an endogenous danger signal.

EXPLORING PATHOGENESIS AND NEW MODELS OF MICROBIAL INFECTION

New data was presented using Drosophila to dissect mechanisms of microbial pathogenesis. For *Salmonella*, Rheinallt Jones (Andrew Neish's lab, Emory University) used transgenic expression of the type III effector virulence gene *AvrA* to find that it suppresses dTAK1, a critical component in IMD-induced NF-κB and JNK pathways. Expression of *AvrA* in the fat body led to inhibition of *Diptericin* expression and increased susceptibility to infection by the Gram-negative bacteria *E. coli* and *Erwinia caratovora*. In the Drosophila eye, expression of *AvrA* suppressed the small and rough eyes caused by overexpression of *dTAK1* or *eiger*. This suggested that *AvrA* was acting at the level of, or downstream of dTAK1. He reported that expression of *AvrA* resulted in inhibition of JNK phosphorylation in the larval fat body. The model presented suggests that *AvrA* from the *Salmonella* results in inhibition of JNK-mediated immune and/or apoptotic responses, although it is possible that *AvrA* also inhibits Relish activation. It will be important to confirm this role for *AvrA* by infecting flies with wildtype and *avrA* mutant Salmonella. Yiorgos Apidianakis (Laurence Rahme's lab, Massachusetts General

Hospital) found that JNK signaling is also important for response to a local infection with *Pseudomonas aeroginosa*. He found that the route of infection influences the host defense responses to the pathogen: infection by pricking the thorax with a bacterial-laden needle caused a local infection, while microinjection of bacteria led to a systemic infection. In the field, labs are split in their preference for thorax pricking vs. microinjection into the abdomen—these data suggest (at least for *Pseudomonas* infection) that the infections and immune responses will differ according to the route of inoculation. Local (thorax) infections modulated muscle gene expression via JNK signaling and this interaction may be key for *P. aeruginosa* virulence. Roberto Ferrarese and Angel Tibbs (Shubha Govind's lab, CUNY) presented data on the interaction between parasitoid wasps (avirulent *Asobara tabida,* and virulent *Leptopilina boulardi-17* and *L. heterotoma-14*) and the Drosophila immune response. The immune response to wasp eggs is primarily driven by a cellular response, with specialized blood cells, lamellocytes, that encapsulate the egg. They found that the Toll and IMD pathways are activated and up-regulated by avirulent *A. tabida* or virulent *L. bourlardi* infection. In contrast, virulent *L. heterotoma* do not induce these humoral responses. The eggs from the two virulent wasp strains are not encapsulated. The parasitoid wasp infection provides a natural system to study the potential coordination between humoral and cellular immune responses, although it is still very unclear if there is any causal relationship.

Alison Fedrow (Keith Chapes' lab, Kansas State University) makes use of S2 cells as a model for mammalian macrophages. She found that *Ehrlichia chafeensis,* an obligate intracellular bacteria that causes human monocytic ehrlichiosis, can propagate in S2 cells and remain infectious to mammalian macrophages. She plans to identify genes important for pathogenesis through RNAi and microarray analyses. Along the lines of the fly as a model mosquito, Robert Glaser (Wadsworth Center, NY State Department of Health) showed data indicating that Drosophila can be infected with West Nile Virus (WNV). WNV is an RNA virus that is carried by mosquitoes and birds, and can cause fever-like symptoms in humans. WNV infection is not lethal to Drosophila, so the viral infection was assayed by titering viral loads. He presented data indicating that an RNAi response is induced against the virus in S2 cells, and mutations in the RNAi pathway (*piwi, spindle-E*) lead to much higher titers of the virus in the fly. This shows the importance of RNAi against West Nile, at least in flies, but also establishes Drosophila as a viable system to identify genes important for susceptibility to WNV in mosquitoes. The power of this approach was demonstrated by Hirotaka Kanuka (Obihiro University, Japan; in collaboration with David Schneider, Stanford University) with their use of Drosophila as a host for Plasmodia parasites, the causative agents for malaria. From a screen in Drosophila, they identified a C-type lectin, *furrowed* (CG1500) as important for the response to *P. gallinaceum*. They present data that RNAi of *furrowed* in *Anopheles gambiae*, the vector for the human malarial pathogen *P. falciparum*, leads to increased *P. berghei* oocysts in the mosquito midgut. Furrowed may represent an important receptor for containing Plasmodia infection in the insect. As such, it may provide a target for manipulating the ability of the mosquito to transmit Plasmodia.

OUT OF THE LAB, INTO THE WILD

Several presentations focused on identifying bacteria that naturally infect flies. Punita Juneja (Brian Lazzaro's lab, Cornell University) captured Drosophila in New York State, then cultured bacteria from the hemolymph of these wild flies and used nested PCR for 16S rDNA to identify bacterial species. She found that the frequency of infection was low (1–2.5%) suggesting that flies in the wild can clear most infections that are not rapidly lethal. The bacteria identified from wild Drosophila included many *Staphylococcus* strains and a lot of soil microbes like *Serratia* and *Pseudomonas*, that are linked to opportunistic infections in humans. When cultured, most of the identified bacterial strains were not lethal to Oregon R flies. One exception (in work presented by Madeline Galac, also of the Lazzaro lab) was new *Providencia* species, a Gram-negative bacteria, that caused 100% mortality within five days. Infection of flies with this *Providencia* also led to decreased antimicrobial peptide gene induction, relative to other infections, suggesting that this microbe may be suppressing or evading the immune response. Vanessa Corby-Harris (Daniel Promislow's lab, University of Georgia) took a similar approach. In this case, ten populations of flies were collected along the East Coast and 16S bacterial rDNA from the fly was cloned and sequenced. Using total fly extracts should also identify bacteria present in the fly gut. This approach enabled her to characterize aspects of each populations' immune responses and determine if this could be correlated with having a diverse bacterial flora. She found that there were lots of γ-proteobacteria (i.e. *Salmonella* and *Pseudomonas*) in the flies. She also found that these 10 populations were variable in their resistance to infection with *Pseudomonas aeroginosa* and *Lactococcus lactis*. She found that there was no correlation with ability to withstand *Lactococcus* infection and microbial richness, but that resistance to *Pseudomonas* correlated with increased microbial richness. It will be interesting to determine whether this resistance to *Pseudomonas* is mediated by the presence of the diverse microbial flora, or if both the flora presence and *Pseudomonas* resistance is due to differences in the immune responses of the different fly populations.

GOING PHYSIOLOGICAL (AND USING THE ORGANISM TO DISCOVER NEW THINGS)

Several very interesting presentations at the meeting came from studies asking different questions about how the fly deals with infection. Linh Pham (David Schneider's lab, Stanford University) described the discovery of an adaptive or priming response in Drosophila.[14] This is dogma challenging, as innate immunity is often defined as *not* being adaptive. She found that if flies were primed with a non-lethal dose of *Streptococcus pneumoniae*, this could protect against a later lethal dose of same bacteria. This priming response was specific, as other pathogenic microbes could not substitute for either the priming or the lethal dose. The ability to specifically prime and prevent a later lethal dose was also seen with infection with the entomopathogenic fungi, *Beauvaria bassiana*. This suggests that the ability to adapt may be a generally important innate immune response to pathogenic microbes. This response was not dependent on an antimicrobial peptide response, but was dependent on the hemocytes. The data presented also indicated that the Toll pathway and the JAK/STAT signaling may play roles in this priming response. Mimi Shirasu-Hiza (also of the Schneider lab) presented an engaging talk on the relationship between infection and circadian rhythms. She found that following a pathogenic infection, flies lose their circadian rhythm and get less sleep. Is losing rhythm helpful in fighting an infection? She found that circadian rhythm mutants, *period* or *timeless*, are more susceptible to *Streptococcus pneumoniae* and *Listeria monocytogenes*, but more resistant to *Burkholderia cepacia*. If the

wildtype flies are infected with a non-lethal dose of *S. pneumoniae*, they lose their rhythm initially, but upon clearing the infection, they can regain their rhythm. This brings up intriguing questions of how our immune system might affect our behavior and vice versa. Definitely something to think about next time you can't sleep—maybe it's an infection that's keeping you up and not worries about getting that grant funded.

CONCLUDING REMARKS

The immunology and pathogenesis research presented at this year's Drosophila Research Conference spanned a wide breadth of topics. Work continues on the IMD and Toll pathways, focusing evermore on the molecular mechanisms involved in signal transduction. New roles for the Drosophila NF-κB homologs in the development of hemocytes and in cellular immunity were described. New models of microbial pathogenesis, with a variety of microbes, were presented, leading to new and surprising insights. And, new natural bacterial pathogens of flies were reported. Overall, this is an exciting time in the field—the foundations of Drosophila immunity, discovered by focusing on the response to few select microbes, are now being applied to more challenging questions, detailing host-pathogens interactions. The host's response to pathogens, and the microbes counter-responses, are being elucidated. In addition, field studies are probing how populations change in response to different pathogen burdens. All these approaches, each of which takes advantage of some of the unique and powerful experimental attributes of Drosophila research, will continue to contribute to this fast moving field.

References

1. Kleino A, Valanne S, Ulvila J, Kallio J, Myllymaki H, Enwald H, Stoven S, Poidevin M, Ueda R, Hultmark D, Lemaitre B, Ramet M. Inhibitor of apoptosis 2 and TAK1-binding protein are components of the Drosophila Imd pathway. EMBO J 2005; 24:3423-34.
2. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, Reinach P, Zang JW, Ge BX. Drosophila TAB2 is required for the immune activation of JNK and NF-κB. Cell Signal 2006; 18:964-70.
3. Geuking P, Narasimamurthy R, Basler K. A genetic screen targeting the tumor necrosis factor/Eiger signaling pathway: identification of Drosophila TAB2 as a functionally conserved component. Genetics 2005; 171:1683-94.
4. Silverman N, Zhou R, Erlich RL, Hunter M, Bernstein E, Schneider D, Maniatis T. Immune activation of NF-κB and JNK requires Drosophila TAK1. J Biol Chem 2003; 278:48928-34.
5. Gesellchen V, Kuttenkeuler D, Steckel M, Pelte N, Boutros M. An RNA interference screen identifies Inhibitor of Apoptosis Protein 2 as a regulator of innate immune signalling in Drosophila. EMBO Rep 2005; 6:979-84.
6. Leulier F, Lhocine N, Lemaitre B, Meier P. The Drosophila inhibitor of apoptosis protein DIAP2 functions in innate immunity and is essential to resist gram-negative bacterial infection. Mol Cell Biol 2006; 26:7821-31.
7. Huh JR, Foe I, Muro I, Chen CH, Seol JH, Yoo SJ, Guo M, Park JM, Hay BA. The Drosophila inhibitor of apoptosis (IAP) DIAP2 is dispensable for cell survival, required for the innate immune response to gram-negative bacterial infection, and can be negatively regulated by the reaper/hid/grim family of IAP-binding apoptosis inducers. J Biol Chem 2007; 282:2056-68.
8. Galko MJ, Krasnow MA. Cellular and genetic analysis of wound healing in Drosophila larvae. PLoS Biol 2004; 2:E239.
9. Bidla G, Lindgren M, Theopold U, Dushay MS. Hemolymph coagulation and phenoloxidase in Drosophila larvae. Dev Comp Immunol 2005; 29:669-79.
10. Tang H, Kambris Z, Lemaitre B, Hashimoto C. Two proteases defining a melanization cascade in the immune system of Drosophila. J Biol Chem 2006; 281:28097-104.
11. Bidla G, Dushay MS, Theopold U. Crystal cell rupture after injury in Drosophila requires the JNK pathway, small GTPases and the TNF homolog Eiger. J Cell Sci 2007; 120:1209-15.
12. Matova N, Anderson KV. Rel/NF-κB double mutants reveal that cellular immunity is central to Drosophila host defense. Proc Natl Acad Sci USA 2006; 103:16424-9.
13. Huang L, Ohsako S, Tanda S. The lesswright mutation activates Rel-related proteins, leading to overproduction of larval hemocytes in *Drosophila melanogaster*. Dev Biol 2005; 280:407-20.
14. Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS. A specific primed immune response in Drosophila is dependent on phagocytes. PLoS Pathog 2007; 3:e26.