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Meeting Report

Insect Immunity: From Pattern Recognition to Symbiont-Mediated Host Defense

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The Jacques Monod conference "Insect Immunity in Action: From Fundamental Mechanisms of Host Defense to Resistance Against Infections in Nature," organized by Ulrich Theopold (Stockholm University, Sweden) and Dominique Ferrandon (CNRS, France), was held in May 2009 in Aussois, France. Here, we review key topics and concepts that were presented and highlight emerging trends in the field of insect immunity.

Introduction

Studies of insect immune responses have had a profound impact on our understanding of how insects fight microbial infections and the nature of metazoan innate immunity. Significant progress has recently been made in research on insect immunity, and there has been a new focus on naturally acquired infections. The Jacques Monod conference "Insect Immunity in Action," organized by Ulrich Theopold and Dominique Ferrandon at Aussois (France) and supported by the CNRS, provided a superb opportunity for researchers in this field to come together and discuss recent developments and the future of research in insect immunity. Here, we will review the key topics and concepts that were illuminated by this workshop. We also wish to pay tribute to Hans G. Boman, a pioneer in the field, who characterized the first inducible antimicrobial peptide in 1981 and sadly passed away in December (Figure 1).

Pattern Recognition: New Insights into Toll Pathway Activation

Unlike mammalian Toll-like receptors, the *Drosophila* Toll receptor does not interact directly with microbial products and is instead activated by a cleaved form of the secreted cytokine-like molecule Spätzle. During the immune response, Spätzle processing results from complex cascades of serine proteases initially activated by secreted pattern recognition receptors sensing Gram-positive bacteria or fungi (Lemaitre and Hoffmann, 2007). New insights into the mechanism of activation of the Toll pathway were presented during the meeting. Activation of the Toll pathway by fungi is, in part, mediated by GNBP3 through the sensing of β(1,3)-glucans. GNBP3 belongs to the β-glucan recognition protein family and contains an N-terminal domain that binds to β(1,3)-glucans and a C-terminal domain that is homologous to the catalytic domain of β-glucanase. Until now, no information has been available concerning the structure of this class of pattern recognition receptor. Alain Roussel (Orléans, France) reported the structure of the N-terminal domain of GNBP3, revealing an immunoglobulin-like fold in which the glucans-binding site is masked by a loop (Figure 2A, box 2). GNBP3 shows specificity of interaction with long-chain polysaccharides, and mutagenesis revealed an essential role for the occluding loop in providing this specificity, highlighting a distinctive mechanism for β-glucan recognition. This work also indicated the importance of accessibility to ligands for pathogen recognition, with the recombinant GNBP3 N terminus binding specifically to new buds and bud scars of *Candida albicans*. Petros Ligoxygakis (Oxford, UK) discussed how a single pattern recognition receptor, the peptidoglycan recognition protein (PGRP) PGRP-SA, can sense Gram-positive bacteria with widely differing cell wall compositions (Figure 2A, box 4). He reported that, in wild-type *Staphylococcus aureus*, fluorescently labeled PGRP-SA binds only to newly generated cell walls during division. However, in a teichoic acid mutant strain, PGRP-SA is able to bind all over the cell wall. This suggests that the presence of teichoic acid in the cell wall can limit the access of PGRP-SA to its ligand, peptidoglycan. This finding perhaps explains previous results that show different requirements for PGRP-SA and PGRP-SD as coreceptors of GNBP-3 for different bacteria, suggesting that different recognition complexes are required for different cell wall compositions. These studies are providing new insights into how pattern recognition occurs in vivo for the detection of live microbes.

Bok Leul Lee (Busan, South Korea) has purified several pattern recognition receptors and serine proteases from the haemolymph of a coleopteran insect, *Tenebrio molitor*, and was able to reconstitute in vitro all of the steps from binding of peptidoglycan to the PGRP-SA/GNBP1 complex, or glucan to GNBP3, to Toll pathway activation by Spätzle (Figure 2A, box 3). He demonstrated that all of these pattern recognition receptors recruit a unique apical modular serine protease (Roh et al., 2009), as recently observed in *Drosophila* (Buchon et al., 2009b). He plans to use these reconstituted pathways to develop kits capable of detecting minute quantities of peptidoglycan or glucan in solution. Studies presented by David Gubb (Derio, Spain) suggested a new and unexpected mechanism to tightly regulate these proteolytic cascades by the scavenging of serpin/protease complexes (Figures 2A, box 1, and 2B). The turnover in the haemolymph of Necrotic, a serpin regulating the Toll pathway, is extremely rapid. Surprisingly, Necrotic is removed from the haemolymph by two groups of giant cells, the garland and pericardial athrocytes, and this endocytic uptake requires the

Figure 1. Hans G. Boman and the Discovery of Antimicrobial Peptides
Having worked previously on tRNA methylation and penicillin resistance, it was in the 1970s that Hans Boman (1924-2008) turned his interest to insect immunity. At the time, the existence of innate immunity was not recognized, and he wondered how insects were able to protect themselves from pathogens? With his coworkers Håkan Steiner and Dan Hultmark, he discovered the first antibacterial peptide, Cecropin, in the *Cecropia* moth in 1981 (Steiner et al., 1981). This seminal discovery opened up a whole new field of research, leading to the isolation of antibacterial peptides from all walks of life and the realization of the importance of innate immunity in the initial response to infections in humans. The discovery of inducible antimicrobial peptides also paved the way to the genetic analysis of their regulation in *Drosophila*.

low-density lipoprotein (LDL) receptor LpR1 (Soukup et al., 2009). This mechanism is reminiscent of the removal of inert serpin/proteinase complexes from circulation in mammals via LDL-receptor-mediated endocytosis in the liver.

Mining the Imd Pathway

Although a number of new pathways have been identified as playing important roles in innate immunity, there is still much to be learned about the Imd pathway (Lemaitre and Hoffmann, 2007). Recent studies have identified several negative regulators of the Imd pathway. François Leulier (Gif-sur-Yvette, France) showed that Pirk (referred to in his work as PIMS) is required to establish immune tolerance to commensal bacteria in the gut (Lhocine et al., 2008) (Figure 2A, box 8). A marked increase in basal Imd activity is seen in *pirk/pims* mutant flies, and this chronic activation seems to be deleterious, with *pirk/pims* mutants showing a reduced life span. Both of these effects are rescued in bacteria-free flies, suggesting that they are dependent on the presence of commensal bacteria. Thus, negative regulation of the Imd pathway is crucial to prevent a chronic immune response to gut bacteria.

Why should there be so many negative regulators of the Imd pathway when no clear negative regulators of the Toll pathway are known? This could relate to the fact that Imd is increasingly being shown to have broader immune roles than Toll, regulating antimicrobial peptides in epithelial tissues as well as systemically. Because only epithelial tissues are constantly exposed to commensal and dietary microbes, it may be that stringent control of the Imd pathway is required in these tissues to prevent excessive basal activity.

In spite of its central role in immunity, the nature of the interactions between components of the Imd pathway is still poorly understood. Neal Silverman (Massachusetts, USA) has been attempting to rectify this situation and reported a key role for ubiquitination in generating a scaffold to assemble pathway components (Figure 2A, box 5). He showed that cleavage of Imd by the caspase Dredd exposes a domain that allows interaction with the putative E3 ligase Diap2. This interaction leads to K63 polyubiquitination of Imd, a modification known to play roles in signal transduction. In this case, Silverman proposes that this polyubiquitination serves as a scaffold to mediate interaction with the downstream pathway components Tab2, Tak1, and the IKK complex. Marie-Odile Fauvarque (Grenoble, France) identified a deubiquitinase, USP36, that is responsible for removing these K63 polyubiquitin chains and is likely to indirectly promote K48 polyubiquitination and degradation of IMD (Figure 2A, box 7). This deubiquitination is required to suppress constitutive activation of IMD and downstream pathways. Thus, the control of the ubiquitination state of IMD is essential to regulation of the pathway.

Activation of the IMD pathway results in cleavage of the transcription factor Relish, allowing its NF-κB module to enter the nucleus. Silverman discussed his finding that phosphorylation of the transcription factor Relish is not required, as previously suspected, for its cleavage. Rather, Relish phosphorylation is specifically required for recruitment of RNA polymerase II to target promoters (Ertürk-Hasdemir et al., 2009) (Figure 2A, box 6).

JAK/STAT as a Tissue Damage Response Pathway

The JAK-STAT pathway has already been identified by gene expression profiling as a regulator of a subset of *Drosophila* immune response genes, but its function was not clear (Agaisse et al., 2003). However, new data serve to emphasize its key role in response to tissue damage. Increasing evidence in vertebrate systems supports the notion that immune responses have a role in tumor surveillance. José Carlos Pastor-Pareja (New Haven, USA) reported the exciting observation that haemocytes (circulating immune cells) adhere to tumors in *Drosophila* upon detection of basement membrane disruption (Pastor-Pareja et al., 2008) (Figures 2A, box 10, and 2C). The same phenomenon is observed in response to physically inflicted wounds that breach basement membranes. In both cases, the damaged tissues upregulate JNK activity and secrete Unpaired cytokines that activate JAK/STAT signaling in haemocytes and the fat body and lead to haemocyte proliferation. These observations suggest that the basement membrane, which surrounds every organ,

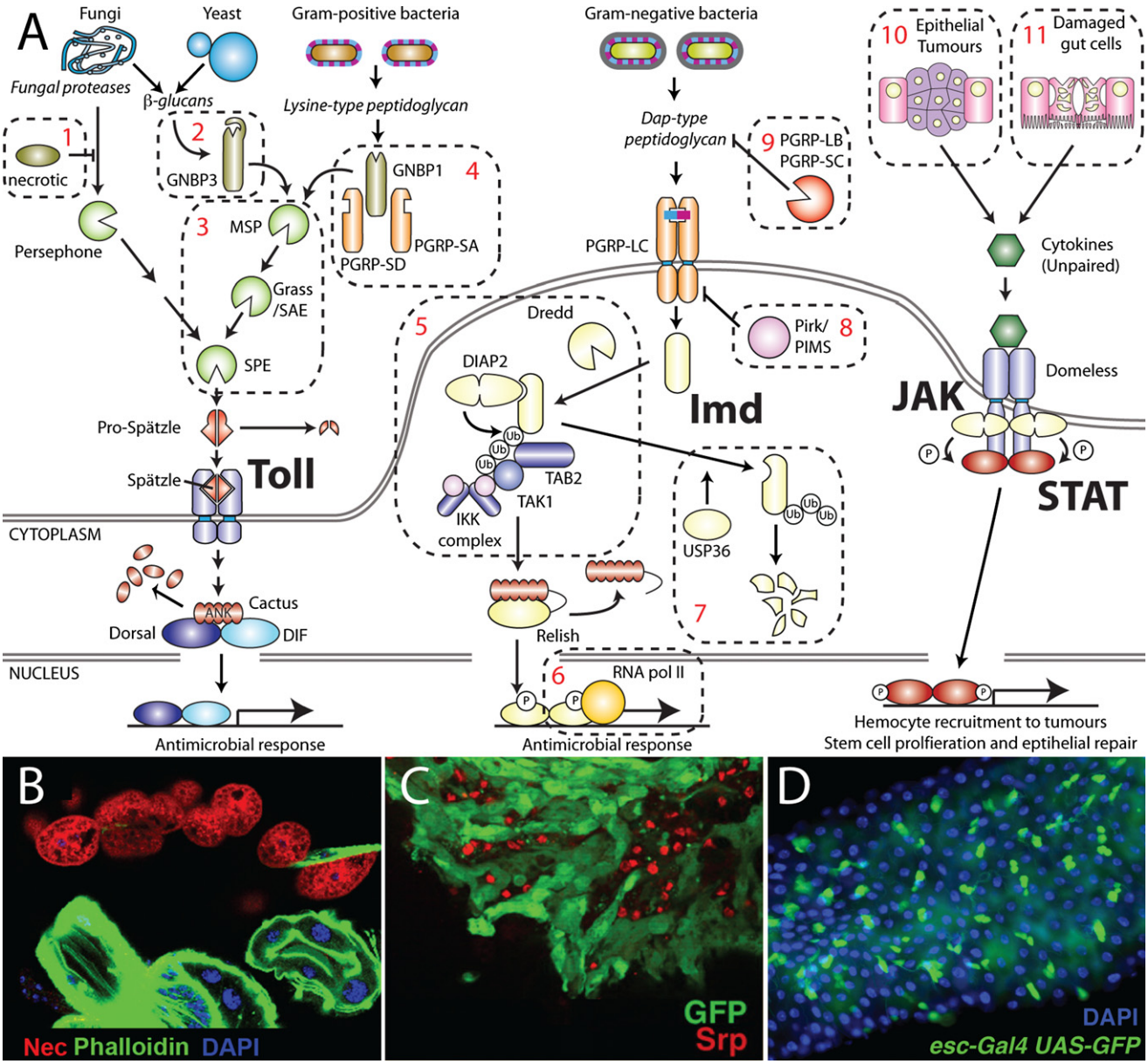

Figure 2. Key Pathways of Innate Immunity, Highlighting New Findings

(A) The Toll pathway is activated in response to Gram-positive bacteria or Fungi, via a cascade of serine proteases leading to the cleavage of the Toll ligand Spätzle. Studies presented at the meeting highlighted the rapid degradation of the serine protease necrotic (1, David Gubb), the structure of the pattern recognition receptor GNBP3 (2, Alain Roussel), the fact that both GNBP3 and GNBP1 activate the same cascade of serine proteases (3, Bok Luel Lee), and the combinatorial nature of the receptors that detect Gram-positive bacteria (4, Petros Ligoxygakis). The Imd pathway detects Gram-negative bacteria and is directly activated by PGRP-LC binding of peptidoglycan. The downstream pathway was clarified at the meeting by Neal Silverman (5 and 6), who showed that Imd is cleaved by the caspase Dredd, allowing it to associate with the E3 ligase Diap2. This results in K63 polyubiquitination, likely forming a scaffold for additional complex members and leading to cleavage and phosphorylation of the NF-κB transcription factor Relish. Relish enters the nucleus and recruits RNA polymerase II to target promoters in a phosphorylation-dependent manner. Other talks discussed the downregulation of Imd signaling by the deubiquitinase USP36 (7, Marie-Odile Fauvarque), by Pirk/PIMS (8, Francois Leulier), and, in the tsetse fly, by PGRP-LB (9, Serap Aksoy). The JAK/STAT pathway is activated by cytokines that bind to the receptor Domeless, resulting in phosphorylation of the transcription factor STAT by the kinase JAK. STAT dimers then translocate to the nucleus and activate a transcriptional response. JAK/STAT plays a role in the response to stress or injury, and work presented at the meeting showed a role for this pathway in the recruitment of haemocytes to tumors and epithelial wounds (10, José Carlos Pastor-Pareja) and the activation of epithelial renewal in response to gut damage caused by infections (11, Dominique Ferrandon, Nicolas Buchon).

(B) Uptake of the serpin protease Necrotic (red) by Garland cells, as reported by David Gubb. Adapted from Soukup et al. (2009).

(C) Recruitment of haemocytes (marked by Serpent in red) to a GFP-expressing $Ras^{V12}/scrib^{-/-}$ tumor in the eye antennal disc, as reported by José Carlos Pastor-Pareja. Adapted from Pastor-Pareja et al. (2008).

(D) Upregulation of cell proliferation in the gut (marked by GFP under the control of the *escargot-Gal4* driver) following oral infection with *Ecc15*, as reported by Nicolas Buchon. Reproduced from Buchon et al. (2009a).

has a general role as an indicator of tissue integrity, allowing the immune system to sense damage by assessing basal membrane status. Further studies should analyze how immune genes regulated by the JAK-STAT pathway participate in wound repair.

Gut Immunity

The systemic immune response of *Drosophila* has been extensively studied, based on a septicemia model of direct introduction of bacteria into the body cavity. However, in recent years, the focus has shifted to the local immune responses of epithelial tissues, particularly the gut, and this was a prominent theme of the meeting.

Damage Sensing and Epithelial Renewal

To maintain homeostasis, the gut epithelium is constantly renewed by the division and differentiation of intestinal stem cells (ISCs). Several recent reports have highlighted an unexpected link between this epithelial renewal and oral bacterial infection. It was recently shown that ingestion of an infectious but nonlethal bacterium *Erwinia carotovora 15* (*Ecc15*) strongly stimulates ISC division, promoting a rapid turnover of the gut epithelium (Buchon et al., 2009a). How this renewal is stimulated and what purpose it serves remained unclear. Nicolas Buchon (Lausanne, Switzerland) presented evidence that increased epithelium renewal is a response to self-inflicted damage of the intestine caused by reactive oxygen species (ROS) produced, in part, by the NADPH oxidase Duox (see below) (Figures 2A, box 11, and 2D). The resultant stem cell proliferation is induced by the JAK-STAT pathway upon the release of the cytokine Upd3 by damaged enterocytes. These data suggest that gut homeostasis is maintained in the face of bacterial infection by balancing the cell damage caused by the protective ROS response with epithelial repair through ISC activation. This is clearly an important facet of gut immune defense, given that mutant flies that are unable to repair their epithelium are highly susceptible to *Ecc15* infection. Dominique Ferrandon (Strasbourg, France) reported the results of an extensive in vivo RNAi screen covering 78% of genes in the genome, which aimed to identify genes affecting survival after oral infection with the lethal entomopathogenic bacterium *Serratia marcescens* (Cronin et al., 2009) (Figure 2A, box 11). More than 800 genes were identified, with overrepresentation of a number of biological processes, including signaling, intracellular transport, and transcriptional regulation. In addition to this technical tour de force, these studies also revealed that *S. marcescens* triggers epithelial renewal through the JAK-STAT pathway. Contrary to observations with *Ecc15*, reducing the capacity for epithelium renewal increased the survival of flies, suggesting that *S. marcescens* subverts host defenses by triggering excessive and ultimately deleterious proliferation of gut cells. Conversely, high doses of another lethal bacterium, *Pseudomonas entomophila*, were reported by Buchon to disrupt the gut by blocking epithelium renewal, suggesting that epithelium renewal could be a common target for manipulation by pathogenic bacteria.

New Pathways in the Regulation of ROS Production

One of the most interesting findings in recent years has been the discovery of the role of the NADPH oxidase Duox in the elimination of ingested microbes. The participation of the NADPH gp91(phox) in the killing of microbes in the phagosomes of macrophages is well known to immunologists. Less clear, however, is the role of a second conserved family of NADPH proteins called Duox proteins. In addition to the NADPH domain, Duox proteins have an N-terminal extracellular peroxidase domain (PHD) that can produce ROS in a regulated manner. The group of Won-Jae Lee (Seoul) has shown that Duox is responsible for a rapid synthesis of ROS in the gut following oral ingestion of bacteria. In the absence of Duox, ingested bacteria are able to persist and proliferate in the intestinal tract, and increased mortality is observed (Ha et al., 2005). Won-Jae Lee (Seoul, South Korea) presented data revealing complex regulation of Duox through two distinct pathways. The activity of the Duox protein is first regulated through a Gαq/phospholipase-Cβ pathway leading to the release of intracellular Ca^{2+} from the endoplasmic reticulum. Under basal conditions, this regulation is essential to control dietary microbes, with even dietary yeast killing flies lacking Duox, Gαq, or PLCβ (Ha et al., 2009). A second level of regulation is the transcriptional upregulation of the *Duox* gene in response to pathogenic microbes through a p38-MAPK pathway downstream of the peptidoglycan receptor PGRP-LC and Imd (but not downstream of Imd pathway components). The PLCβ pathway is required both to downregulate *Duox* expression in basal conditions (via Calcineurin-B and a MAP kinase phosphatase) and as an upstream component of the p38-MAPK pathway to upregulate *Duox* expression in response to pathogenic microbes. This suggests a crucial role for the level of PLCβ activity, presumably dependent on the level of the unidentified ligand of this pathway. Clearly, the identity of this ligand (possibly related to stress) and, indeed, the receptor upstream of Gαq (likely a G-coupled receptor) will be important to identify in the future. These studies reveal new mechanisms of microbial killing and new regulatory pathways. Could this role of Duox be conserved in mammalian epithelia? Preliminary studies from the Lee laboratory suggest that a Duox protein, regulated by a PLCβ, is involved in ROS production in cultured mammalian intestinal cells. Along these lines, a recent study in zebrafish revealed a different role for Duox in epithelial cells, in wounding-dependent generation of a gradient of H_2O_2 required for leukocyte recruitment to the wound (Niethammer et al., 2009).

A further suggestion of conservation is seen in the p38-MAPK pathway, which has also been implicated in immune defense in other organisms, such as nematodes and plants. Johnathan Ewbank (Marseille, France) spoke about his work on the regulation of an antimicrobial peptide gene, *nlp-29*, in *Caenorhabditis elegans* (Ziegler et al., 2009). This regulation requires G protein signaling, a PKCδ, and p38. Could this pathway, at the frontier between stress response and immunity, be an ancestral-microbe-sensing system, preceding the emergence of a pattern recognition mechanism?

Trapping and Killing of Bacteria by Clotting

The mechanisms of clotting are poorly conserved between vertebrates and insects, and the only enzyme that is known to play a role in clotting in both groups is transglutaminase (corresponding to vertebrate factor XIII), which crosslinks clotting fibers. Using RNAi, Ulrich Theopold (Stockholm, Sweden) reported that transglutaminase (and clotting in general) is not critical for proper wound healing in flies. Instead, it plays an immune role, trapping and killing bacteria, with transglutaminase

substrates being incorporated into bacterial and fungal cell walls. In the absence of transglutaminase, apparently normal clots form, but these no longer trap bacteria, and larvae are more susceptible to septicemia. This resembles the role of human factor XIII in trapping and eliminating bacteria. That this is a common mechanism for bacterial killing is suggested by the observation of fibrous extracellular traps generated by neutrophils (Wartha et al., 2007). It will be interesting to discover whether *Drosophila* clots contain embedded antimicrobial molecules in the same way as these traps.

Antimalarial Response in *Anopheles*

Plasmodium development within *Anopheles* mosquitoes is a vulnerable step in the transmission cycle of the malaria parasites. Scientists are interested in understanding the interactions leading to this bottleneck, as targeting this step represents a promising strategy for malaria control.

A Complement-like System in Insects

The thioester-containing complement C3-like protein TEP1 and two leucine-rich repeat (LRR) proteins, LRIM1 and APL1C (or LRIM2), have been identified as major factors regulating the *Plasmodium* parasite load in mosquitoes. Recent studies now indicate that these factors function together in a common complement-like pathway. The two LRR proteins circulate in the haemolymph as a multimeric complex held together by disulfide bridges. This complex interacts with and stabilizes the mature form of TEP1. Upon *Plasmodium berghei* infection, TEP1 is released from the complex and binds to the surface of midgut-invading *Plasmodium* parasites, targeting them for destruction. Results presented by Elena Levashina (Strasbourg, France) and George Christophides (London, UK) showed that LRIM1 and APL1C are required for binding of TEP1 to the malaria parasites (Fraiture et al., 2009; Povelones et al., 2009). Depletion of the LRR proteins from the mosquito haemolymph by RNAi results in nonspecific deposition of TEP1 on *Anopheles* tissues, thereby depleting mature TEP1 from circulation in the haemolymph and abolishing its binding on *Plasmodium*. Thus, these major antiparasitic factors in mosquitoes jointly function as a complement-like system in parasite killing.

Some species of *Anopheles* mosquitoes do not serve as vectors for *Plasmodium*, as they always mount a successful immune response against the parasites, and these species provide another way to understand *Plasmodium* resistance. Silencing of *LRIM1*, *LRIM2*, and *TEP1* in the resistant mosquito *An. quadriannalatus* completely abolishes the killing of *P. berghei* by melanization and dramatically increases the number of oocysts, thus transforming it into a highly permissive parasite host (Habtewold et al., 2008).

Role of the Microbiome and Innate Immune Pathways

Anopheles gambiae antimicrobial responses are largely controlled by the Toll, Imd, and JAK-STAT immune pathways via the NF-κB transcription factors REL1 and REL2 and STATs. Different pathways seem to mediate resistance to different parasite species, with the Imd pathway crucial for resistance to the human malaria parasite *P. falciparum*, whereas the Toll pathway appears to be most efficient against the rodent parasite *P. berghei*. In addition to mosquito immune responses, recent studies implicate the microbial gut fauna in *Plasmodium* parasite resistance. To better understand the molecular interplay between commensal bacteria and *P. falciparum* in *An. gambiae*, George Dimopoulos (Baltimore, USA) has adopted a functional genomics approach. He reported that the presence of microbiota in mosquitoes results in the upregulation of a significant subset of immune genes, including several anti-*Plasmodium* factors, and that elimination of the microbiota results in an increased susceptibility to *Plasmodium* infection. Transcriptome analyses suggest that the commensal bacteria modulate mosquito susceptibility to *Plasmodium* through immune responses, plausibly through activation of basal epithelial immunity (Dong et al., 2009). A complementary approach by George Christophides (London, UK) showed that the immune response against midgut microbiota is mediated, at least partly, by the peptidoglycan recognition protein, PGRP-LC. Upon recognition of bacteria, this receptor triggers activation of the REL2-signaling pathway that consequently modulates infections with *Plasmodium*. It is hoped that these studies will allow researchers to boost mosquito defenses against *Plasmodium*, perhaps by producing transgenic mosquitoes or introducing specific bacteria into mosquito populations, and ultimately halt the spread of malaria.

Symbiotic Bacteria and Host Immunity

In addition to their microbiota, at least a third of arthropod species harbor maternally inherited bacteria that live within host cells. New insights into the advantages that these microbes can confer on their hosts and the mechanisms that allow these microbes to escape host immune responses were provided at the meeting.

Among the symbiotic organisms that have been most extensively studied is *Wolbachia*, which infects more than 20% of insect species. Until recently, it was thought that *Wolbachia* infections of insects were largely parasitic and had invaded host populations by manipulating the reproduction of their hosts to increase their transmission through the female germline. However, new studies are suggesting that *Wolbachia* infections in *Drosophila* can confer resistance to viruses and therefore act as mutualists. Independent investigations reported by Karyn Johnson (Brisbane, Australia) and Luis Teixeira (Oeiras, Portugal) have shown that *Drosophila* infected with *Wolbachia* are less susceptible to the mortality induced by a range of RNA viruses, whereas animals cured of *Wolbachia* by tetracycline treatment fully regain their susceptibility (Hedges et al., 2008; Teixeira et al., 2008). *Wolbachia* increases the survival of flies infected with four different RNA viruses—*Drosophila* C virus (DCV), Cricket paralysis virus (CrPV), Nora virus, and Flock house virus (FHV) —but has no effect on survival to infections of the DNA virus insect iridescent virus 6. The increase in resistance against three of these viruses results from a reduction of the viral titer. However, Teixeira showed that titers of FHV are not affected, suggesting that *Wolbachia* confers a tolerance, rather than a resistance, to the virus. Johnson also presented new data showing varying levels of antiviral protection with different *Wolbachia* strains in *Drosophila simulans*. The mechanism(s) by which *Wolbachia* confers protection to infected individuals remains unknown. Given that natural viral pathogens of *D. melanogaster* are common in wild populations, the association of *Wolbachia* with a robust antiviral effect may confer an advantage to flies. Increasing antiviral immunity in vector insects such as mosquitoes, which transmit devastating pathogens like Dengue and West Nile virus, would clearly be of immediate interest, and

Wolbachia presents an alternative strategy to transgenic approaches. This is particularly exciting, as *Wolbachia* can be transferred between species and can rapidly spread through natural populations by manipulating their hosts' reproduction.

Some insects have obligate symbionts, such as *Buchnera* in aphids and *Wigglesworthia* in tsetse flies, that support vital host physiological functions. Studies in tsetse flies by Serap Aksoy (Yale, USA) suggest yet another role for obligate symbiosis in host biology. Adult tsetse flies are highly resistant to infections with parasitic African trypanosomes, but removal of *Wigglesworthia* by antibiotic treatment causes the flies to become highly susceptible to parasitism (Pais et al., 2008). RNAi depletion of a host peptidoglycan recognition protein similar to PGRP-LB suggests that it plays a dual role in tsetse. In *Drosophila*, PGRP-LB has been shown to cleave peptidoglycan into fragments that are not detected by the immune system, thereby downregulating the immune response (Figure 2A, box 9). In tsetse flies, PGRP-LB also seems to scavenge peptidoglycan to prevent induction of immune pathways in response to *Wigglesworthia.* It might also play an additional role in interfering with parasitism. *PGRP-LB* silencing induces the Imd pathway, resulting in the synthesis of antimicrobial peptides, damage to *Wigglesworthia* (Wang et al., 2009), and a consequent loss of host fecundity. At the same time, the flies become highly susceptible to trypanosome parasitism. It is not clear whether this results from a direct antiparasitic function of PGRP-LB or from interference with the synthesis of unknown downstream molecules. Thus, the obligate nature of *Wigglesworthia* symbiosis may result from its beneficial effects on host immunity as well as fecundity.

Employing PGRP-LB to ensure tolerance of obligate symbionts is not isolated to tsetse flies, with a similar role being shown in weevils (Anselme et al., 2006). Wherever symbionts are necessary for the survival or reproduction of their hosts, we might expect to find host mechanisms to suppress immune responses and ensure tolerance of these symbionts. An extreme example can be found in aphids, where genome sequence data indicate extensive erosion of genes and pathways coding for host immune functions (Pennisi, 2009), presumably to protect their obligate symbiont, *Buchnera*.

The Evolution of Resistance and Tolerance

Pathogens can have dramatically different effects on the health of the individuals that they infect. Whereas some pathogens are lethal, others can be relatively benign. For example, Dan Hultmark (Umea, Sweden) described how the recently identified Nora virus can be present in massive quantities in *Drosophila* with only limited consequences (a modest reduction in life span) (Habayeb et al., 2009). Intriguingly, although most flies can clear the virus if maintained in clean conditions (the virus is transmitted through feces), a subpopulation remains persistently infected.

It is increasingly becoming clear that "tolerance" to pathogens (limiting the health impact of a given pathogen burden) may also be a viable immune strategy, reducing the damage caused by the pathogen, rather than mounting a "resistance" response that kills or controls the pathogen. Although the concepts of resistance and tolerance are widely used in the plant sciences, until recently, they have been largely ignored in the study of animal diseases. Several talks focused on the genetic basis of tolerance to infection in *Drosophila*. David Schneider (Stanford, USA) found that a mutation in *Serine protease 7* (*Sp7*), which encodes a component of the melanisation pathway, reduced the survival of flies infected with *Listeria monocytogenes*, despite having no effect on the number of bacteria (Ayres and Schneider, 2008). However, when *Sp7* mutant flies are infected with other species of bacteria, both increased and decreased bacterial load are observed, showing that genes that control tolerance to one pathogen may control resistance to others. Another way in which flies can tolerate infection was described by Naoaki Shinzawa (Obihiro, Japan), who found that overexpression of p38 MAP kinase increased the survival of flies infected with intracellular bacteria, despite having little effect on bacterial load. This effect was caused by the tolerant flies having enlarged plasmatocytes (phagocytes), which prevented phagocytosed bacteria from escaping and harming their host. Understanding how animals can tolerate infection may have important implications for public health. For example, if medical interventions or selective breeding of animals increases tolerance to infection rather than resistance, there will be little selection for pathogens to evolve countermeasures to the increased tolerance. Therefore, such strategies may avoid many of the problems that result from pathogens evolving to survive drug treatments or to infect resistant breeds of animals and plants.

One of the reasons that animals may evolve tolerance rather than resistance is that resistance can be very costly. An overactive immune response can be severely deleterious for an animal, but this effect is only seen after an animal becomes infected. However, increased resistance to pathogens may also be costly in uninfected animals. This was illustrated by Yixin Ye (Queensland, Australia), who described how populations of *Drosophila* that were artificially selected to survive infection with *Pseudomonas aeruginosa* rapidly evolved resistance to the bacterium (Ye et al., 2009). However, this increase in resistance was accompanied by a reduction in the survival and egg hatch rates of uninfected flies. Once selection was relaxed, the population lost its resistance, suggesting selection for susceptibility in the absence of the pathogen.

In contrast to most other organisms, researchers working on *Drosophila* immune responses typically rely on microbes that do not occur in wild flies, and the natural pathogens and parasites of flies are largely unknown. Darren Obbard (Edinburgh, UK) described how both wild and laboratory *Drosophila* are infected with a diverse range of viruses, some of which occur at a high prevalence. These natural pathogens will impose a strong selection pressure on flies to evolve resistance. Frank Jiggins (Cambridge, UK) has resequenced most of the genes in the *Drosophila* immune system in various *Drosophila* populations to investigate how natural selection has acted on them. A small subset of genes in the immune system has a very high rate of adaptive evolution, and these genes tend to be in immune-signaling pathways or to be components of the antiviral RNAi response. Signaling pathways and the RNAi machinery are common targets of pathogen molecules that suppress the immune response, suggesting that immune suppression may be a key force driving evolution of the immune system. The way in which natural selection acts on the *Drosophila* immune system is often strikingly different from the patterns seen in vertebrates. For example, there is no evidence of natural selection maintaining polymorphisms like those seen in vertebrate MHC genes, and vertebrate antimicrobial

peptides often have high rates of adaptive evolution, whereas *Drosophila* ones do not. This is probably the result of insects and vertebrates differing in their ecology, the pathogens they encounter, and their immune systems.

Perspectives

Though it is not possible to discuss all of the talks presented at this meeting, we hope to highlight emerging trends in the field of insect immunity. One of these is a new interest in the host response to natural infections, focusing on the immune response and homeostasis of the gut. In this context, outcomes vary drastically, depending on the bacterial strains used to infect the fly. This is also a feature of new studies that emphasize the role of tolerance in evading the harmful effects of infections. In some cases, tolerance leads to persistent infections, such as that of the Nora virus, which provide an opportunity to investigate natural transmission of infections between individuals. Many researchers are now analyzing the survival of insects to microbial infection in a more integrated manner that encompasses not only immune defense, but also critical aspects of the host's physiology. Also of interest, and little studied as yet, is understanding the underlying causes of death following pathogenic infections. Massive cell death of the intestinal epithelium seems to underlie the lethality of some oral infections, and Ioannis Eleftherianos (Strasbourg, France) presented data suggesting that infection with the Flock house virus may be causing death through heart failure, with a role for stress-activated potassium channels in mediating survival.

A much-increased emphasis is also being placed on the role of nonpathogenic microbes in immune defense, including both the microbiota and symbionts, which in many cases enhance immunity or even provide novel immune functions. Of particular interest are the parallels between mechanisms employed during interactions with pathogens and symbionts. This is clearly illustrated by the case of catalytic PGRPs, which seem to play roles in moderating the immune response to pathogens and allowing tolerance of symbionts. Finally, an intriguing case was made for the importance of social interactions in immunity focusing on the social insects, the ants, in which a "social immune memory" was proposed by Sylvia Cremer (Regensburg, Germany) to explain increased survival to infection of naive ants exposed to infected colony members, and bees, concerning which Jay Evans (Beltsville, USA) discussed colony level defenses and the pathogens associated with Colony Collapse Disorder.

Thus, research in the insect immunity continues to be a rich and diverse field, having an impact on our general understanding of metazoan immunity, and also has the potential to improve human health through innovative strategies to control or manipulate insect disease vectors.

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