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Epilogue: Pathways into the Future of Insect Immunology

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Insect immunology is a critical subject relevant to insect pathology, parasitology, insect pest control, and human health that interfaces with many areas of insect biology and ecology. Potentially fruitful areas of future research in insect immunology have been identified throughout the preceding chapters of this book. Identification of signaling pathways critical to non-self recognition of parasites and pathogens represents an especially promising field that challenges future investigators, as these pathways are critical to determining whether the biotic agent is virulent or avirulent to the host. The dynamic interplay between virulence genes of the pathogen and gene(s) encoding factors responsible for susceptibility or resistance of the host provides evidence for the evolution of complex strategies of molecular host–pathogen and host–parasite interaction in many insect systems. While such relationships are often described as representative of a co-evolutionary arms race taking place between the host and its invader, the evidence accumulated thus far suggests that pathogens and parasites can manipulate their relationship with the host to establish a balanced equilibrium between pathogen virulence characteristics and host resistance mechanisms which culminates in successful infection of susceptible hosts.

Recent studies of the social Amoeba *Dictyostelium discoideum* have revealed that these evolutionarily primitive organisms have cells capable of non-self recognition and defense. The presence of functional immune 'sentinel cells' in these species, representing some of the first multicellular organisms of ancient origin, argues that immunity is a basic property of organismal biology. Hence, many features of insect immunity likely had their origins within simple multicellular organisms. Continued focus on comparative immunological analyses are very likely to reveal how these components, and the regulatory pathways they mediate, co-evolved on a temporal scale in both invertebrate and vertebrate phyla.

The evolutionary relationships and physiological interface between insect and mammalian immunity are especially complex in insect vectors that feed on vertebrate blood. Moreover, the bloodfeeding behavior of insect vectors of human and animal diseases offers a wealth of potential opportunities for controlling rates of disease transmission among vertebrate hosts. To achieve this goal, a better integration of the study of mammalian and arthropod immunity is not only logical, given the contribution of insects to our understanding of mammalian innate immunity, but also an absolute necessity, given the immunological interface of bloodfeeding. The physiological juxtaposition of vertebrate and insect immune systems in such relationships provides strong evidence for the evolution of sophisticated strategies of physiological interaction between two evolutionarily distinct taxonomic animal groups. To reduce rates of disease transmission, there are two opportunities to manipulate this bloodfeeding interface: at the point of feeding via injection of vector saliva into the vertebrate host, and within the gut of the arthropod vector to block the movement of the parasite/pathogen into the hemocoel and its ultimate transfer to the salivary glands. The conserved signaling pathways and effectors in vector species provide tremendous insight into the evolution of immunity, a fact that receives little attention in most immunology courses. However, this conservation also facilitates a surprisingly complex immunological communication between these biologically disparate systems. Dissection of this biology to identify the 'master switches' will probably require a computational or systems biology approach. From an applied perspective, manipulation of these interfaces provides a basis for strategies to disrupt pathogen transmission at multiple levels. Thus, to study the immunology of arthropods and their mammalian hosts in isolation overlooks the basic fact that they are intricately connected through bloodfeeding.

Additionally, a survey of vertebrate host immunological responses to insect vector feeding activity provides ample opportunities to curb rates of disease transmission among susceptible individuals via enhancement of immunological reactivity in the vertebrate host. As described above, manipulation of the vector's immunological responses to parasitic or pathogenic infection can result in enhancement in levels of vector refractoriness to the disease agent, thereby reducing rates of disease transmission. Identification of genetic components contributing to vector refractoriness will have a similar beneficial effect on reducing vertebrate infection rates as these critical genes provide avenues for manipulation of vector competence.

How pathways of insect immunity are regulated by cross-talk from other regulatory systems in the host can form the foci for new areas of research that will also potentially impact agriculture and disease transmission. For example, interactions between the immune and nervous systems of insects are only now just beginning to be addressed as a new subfield of insect immunology. At the moment, we lack a clear understanding of the details of immune-neural connections at all levels in insect systems. While psychoneuroimmunology is already an established field in vertebrate (particularly mammalian) immunology, such links have proved difficult to decipher in insects. The chemical identity of the cytokines that are the major players in altering neuronal function in insects, and assessment of whether they cross the insect blood–brain barrier, remain open questions. Isolation of the neuronal receptors for these cytokines, as well as characterization of the subset(s) of neurons responding to specific molecules, in the insect host also remain subjects for future study.

In the other direction (neural to immune), we lack any direct evidence that the nervous system has a direct effect on insect immune function (e.g. via neural connections to immunologically responsive tissues such as fat body). Integration of information from both insect neurobiology and immunology will be critical to understand the biological significance of immune function changes induced by neural substances such as octopamine. Thus, our base of knowledge about insect neuroimmunological interactions is still in its infancy and a variety of new experimental approaches will be required to tease these complex but crucial relationships apart.

Similarly, the impact of putative hormonal factors on host immunocompetence has been a subject of much scientific speculation but minimal experimentation thus far in insect biology, although such influences are well-established in vertebrates (e.g. the critical roles of sex hormones in regulating vertebrate host immunity). In holometabolous insects, older instars of insect larvae often display enhanced resistance to viral and bacterial pathogens, as well as parasitoids, compared to younger developmental stages, suggestive of a possible hormone-mediated regulation of immunocompetence. Both juvenile hormone and ecdysteroids are candidate modulators of immunity, as hemolymph levels of these hormones fluctuate in amount in correlation with the insect's stage of development and the temporal progression of the molt cycle. Whether peptide hormones also play regulatory roles in insect humoral and/or cellular immunity is another question, which merits experimentation by future physiologists.

Studies of relationships between insect host behavior and immunity have given rise to the newly emerging field of evolutionary immunology, which frames insect immunity in an ecological context. This new area poses novel challenges for future investigators that have not been presented in this book, which focuses on physiological and molecular aspects of immunity. The fitness costs incurred by mobilization of immune responses to pathogens and parasites have seminal relevance to ongoing efforts to develop strategies of immunologically based manipulation of vector refractoriness to reduce rates of vertebrate disease transmission. While the expression of refractoriness-related gene products in transgenic insects has yet to be implemented successfully in field populations of vectors to control disease agents, we are possibly not far in time from ultimately achieving this goal. The ecological impacts of different aspects of insect immunity alone could form the focus of a separate book on evolutionary immunology to draw new investigators with both basic and applied perspectives to weigh the balances between the fitness trade-off costs and benefits of mobilizing effective immune responses to fight infection which impact fitness traits, such as longevity and reproductive output.

While the initial conception of a gene-for-gene co-evolutionary arms race operating between a host and its invaders had its origins in the field of plant pathology, the dynamics of the juxtaposition of virulence characteristics of pathogens *vis a vis* the resistance traits of the host are similar on molecular as well as organismal levels in both insects and plants. Many features of the virulence traits shown by insect pathogens and parasites have parallels in plant pathology, even though the molecular mediators of virulence and resistance are quite different in these two diverse groups of organisms.

The genetic contributors to determining whether a given insect species or strain is susceptible to a particular parasite or pathogen can frequently be identified by studying genes and gene products expressed in semi- or non-permissive host species that successfully resist infection. While this has been done superbly in *Drosophila* due to the accumulated wealth of genetic and genomic studies focusing on this species for decades, the time is now ripe for characterization of these elements in other agriculturally important host species due to the rapid expansion of genomic and proteomic information for a taxonomically diverse range of insect species. Additionally, while the malaria parasite, *Plasmodium falciparum*, was the first for which the complete genomic sequences of the parasite, vector, and human host were first made available to researchers, the expected completion of full genome sequences for other vector species will facilitate future identification of genes contributing to susceptibility and resistance traits of vectors that transmit parasites and pathogens that threaten human health.

While immunological memory and specificity are usually considered to be two defining characteristics of mammalian immunity, recent studies have pinpointed similar processes operating in insects as described in this book. The phrase 'immunological memory' is often interpreted as synonymous with 'antibody production' in the biological literature, but we now know that insects have evolved different strategies to cope with repeated infections. Although insects lack antibody-mediated responses *per se* they have evolved the capacity to exhibit 'immune priming' and increased tolerance following repeated exposure to an infectious agent, analogous to effects induced by 'vaccination' of the host. Future emphasis on deciphering how this process operates in insects on a mechanistic level will yield more comprehensive understanding of the evolutionary development of host defense pathways throughout the animal kingdom. The use of microarrays and even newer technologies will facilitate immunological specificity studies, to generate surveys of anti-parasite, anti-parasitoid, and anti-pathogenic immune responses of insects and make comparisons amongst the different classes of defenses mobilized against a particular type of infection. While the molecular aspects of memory and specificity of immunity are not yet clearly defined in insects, in contrast to mammals, the similarities reported thus far yield additional insights into the analogous strategies of molecular interaction evolved in insects and vertebrates.

While the utility and relevance of using insect models to study vertebrate immune mechanisms has not yet been fully appreciated by immunologists focusing on disease etiology in humans, many parallels certainly exist. For example, many developmental signaling molecules and pathways play dual immunological roles in insects, and homologous molecules and regulatory processes occur in many vertebrates including humans. The use of insect systems as mammalian disease models offers a plethora of opportunities for unraveling complex modes of physiological and molecular interaction among parasite, pathogen, and host.

Future biochemical studies of insect immunity will also uncover new classes of molecules not previously thought to play immunological roles. While insect lipids and lipid-carrying molecules were previously hypothesized to play a primarily metabolic role, their tandem role in immunity as demonstrated by recent experimental work is illustrated in this book. Our knowledge and identification of potentially important insect cytokines is a field still in its infancy compared to the status of this subfield of vertebrate immunology, and future research emphasis on identification of insect cytokines is likely to prove especially fruitful in isolation of biologically active molecules that can influence host susceptibility versus resistance to parasitism and disease.

Insect parasitoids are unusual parasites in that their presence within the host invariably causes premature host death, thus preventing host reproduction and proliferation of potentially highly susceptible hosts. A broader understanding of the molecular interactions responsible for successful parasitism of a particular host species or strain, as well as the physiological components contributing to definition of a parasitoid's host range, will dramatically increase the potential for successful implementation of biological control programs based upon the exploitation of parasitoids to control expanding populations of pest species. Additional insights into molecular strategies involved in evolution of host resistance to a given insect parasitoid species will also enhance such programs by pinpointing potentially critical host–parasitoid immunological elements and processes that facilitate successful parasitism of pest species. Continued advances in utilization of genomic and proteomic approaches to study the roles of polydnaviruses produced by hymenopteran parasitoids in manipulating lepidopteran host immunity to infection is but one example of how future research directions can impact biotechnological developments in insect pest control. These goals can be attained via expression of immunologically active polydnavirus gene products targeted to the host with implications for their potential exploitation in insect pest associations with pathogens, parasites, and plants.

Deciphering which genes and gene expression products confer disease susceptibility versus resistance also offers exciting opportunities for development of novel biopesticides and a range of biologically based strategies to reduce populations of pest insects. Disease resistance is especially critical to the health of beneficial insects including many parasitoids, predators, and pollinators that play crucial beneficial roles in agriculture. Development of transgenic plants and generation of recombinant biopesticides expressing genes encoding insect-active virulence factors to target growth and development of insect pests is in progress in many countries around the globe, with beneficial economic impacts. Genetic modification of foods or other economically important products remains controversial in several countries due to their as yet not fully documented effects on environmental and human health. Nevertheless, implementation of well-designed bioengineering technologies will continue to expand the approaches available in our arsenal to increase the efficiency of agricultural production and output, to ultimately beneficially impact human and animal nutrition and health. Similarly, expression of immunologically active molecules in transgenic insect vectors offers fresh opportunities to eradicate global disease transmission by short-circuiting the life cycle of the pathogen in the insect, although this strategy has not been widely implemented as yet.

As the chapters in this book have revealed, many new pathways into the future of insect immunology are now challenging us as we focus on both near and far horizons in this field. Pattern recognition processes, the molecules involved in nonself recognition, and signaling pathways that mobilize cellular and humoral defenses, are three topics of current focus that will continue to engage insect physiologists and molecular immunologists for decades to come. The formidable power of genomic and bioinformatic approaches has produced massive assemblages of immunological information about insect systems in just the past 5 years. As we project forward into the future, this rapid pace of progress is anticipated to continue as we introduce many new generations of investigators to the exciting and dynamic discipline of insect immunology.