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Insect immunology and hematopoiesis

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ABSTRACT

Insects combat infection by mounting powerful immune responses that are mediated by hemocytes, the fat body, the midgut, the salivary glands and other tissues. Foreign organisms that have entered the body of an insect are recognized by the immune system when pathogen-associated molecular patterns bind host-derived pattern recognition receptors. This, in turn, activates immune signaling pathways that amplify the immune response, induce the production of factors with antimicrobial activity, and activate effector pathways. Among the immune signaling pathways are the Toll, Imd, Jak/Stat, JNK, and insulin pathways. Activation of these and other pathways leads to pathogen killing via phagocytosis, melanization, cellular encapsulation, nodulation, lysis, RNAi-mediated virus destruction, autophagy and apoptosis. This review details these and other aspects of immunity in insects, and discusses how the immune and circulatory systems have co-adapted to combat infection, how hemocyte replication and differentiation takes place (hematopoiesis), how an infection prepares an insect for a subsequent infection (immune priming), how environmental factors such as temperature and the age of the insect impact the immune response, and how social immunity protects entire groups. Finally, this review highlights some underexplored areas in the field of insect immunobiology.

1. Introduction

Insects interact with a wide array of pathogens. Many of these pathogens seek to invade and colonize the insects they come in contact with, and in many cases, successful colonization leads to detrimental effects to the host. A diversity of pathogenic organisms can infect insects, including viruses, bacteria, fungi, protozoans, nematodes, and even other insects (Mahy, 2004; Pennacchio and Strand, 2006; Vega and Kaya, 2012). Some of these interactions are general whereas others are highly specific. For example, the fungus *Beauveria bassiana* is a facultative pathogen that infects insects from numerous taxonomic orders whereas the *Plasmodium* sp. that cause human malaria are obligate pathogens that only infect – and are only transmitted by – select species of anopheline mosquitoes (Manguin et al., 2008; Ortiz-Urquiza et al., 2015).

To reduce the probability of infection, insects have evolved physical barriers that keep pathogens from entering their main body cavity, or hemocoel. The most encompassing physical barrier that prevents the entry of pathogens is the cuticle (Lundgren and Jurat-Fuentes, 2012; Siva-Jothy et al., 2005). This chitinous, hydrophobic material forms the exoskeleton of the insect, and also lines the foregut, the hindgut, and the tracheal system. Pathogens that enter the body through the cuticle do so through natural wounds, or by the enzymatic digestion of this material. Pathogens also enter the body via ingestion. Following ingestion, pathogens are immediately subjected to antagonistic barriers, such as cibarial or pharyngial armatures, enzymes of the digestive system, inhospitable pH, and the endogenous microbiota (Cirimotich et al., 2011; Lundgren and Jurat-Fuentes, 2012; McGreevy et al., 1978; Siva-Jothy et al., 2005). Ingested pathogens that seek to exit the digestive tract and gain entry into the hemocoel must also traverse the cellular epithelium of the midgut, and in some cases, a non-cellular and chitinous peritrophic matrix (Kato et al., 2008; Kuraishi et al., 2011; Weiss et al., 2014).

Although entering the hemocoel is a formidable enterprise, many organisms have evolved mechanisms to efficiently accomplish this. Some fungi enter the hemocoel by enzymatically degrading the cuticle (Pedrini et al., 2007), and pathogens that are routinely ingested have evolved innovative strategies to exit this intestinal compartment. For example, the bacterium, *Bacillus thuringiensis*, produces Cry toxins that destroy the epithelial cells of the digestive tract, the protozoan parasites that cause malaria utilize their apical complex to penetrate the cells of the midgut, and filarial nematodes physically burrow out of the intestinal space (Christensen and Sutherland, 1984; Roberts et al., 2013; Vachon

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et al., 2012). Some pathogens elect to not leave the gut. For example, *Trypanosoma cruzi* and *Yersinia pestis*, which in humans cause Chagas' disease and plague, respectively, remain within the digestive tract of their kissing bug and flea hosts (Roberts et al., 2013).

Pathogens that come to reside in the midgut, the hemocoel or the internal organs, elicit immune responses that have evolved to eliminate or control infections. These immune responses range from cellular events such as phagocytosis, to humoral events that include lysis and melanization. This review presents an overview of these and other immune processes, and how the primary immune cells of insects, the hemocytes, are produced and replenished.

2. Anatomy of the insect immune system

Once pathogens gain entry into the body of an insect they come to reside in the midgut, the hemocoel, or other cells and organs. The recognition of pathogens as both foreign and dangerous induces immune responses that are driven by multiple types of immune cells and tissues (Fig. 1).

The primary immune cells in insects are the hemocytes (Hillyer and Strand, 2014; Strand, 2008). These cells, which are present in the hemocoel, drive cellular immune processes such as phagocytosis, and produce humoral immune factors that lead to pathogen killing via lysis or melanization. Hemocyte populations can be divided using two, non-exclusive criteria: their spatial state and their functional properties. From a spatial perspective, hemocytes either circulate with the hemolymph, in which case they are called circulating hemocytes, or are attached to tissues, in which case they are called sessile hemocytes (Hillyer and Strand, 2014; Strand, 2008). The distinction between circulating and sessile hemocytes is strictly spatial, and sessile hemocytes can release from their point of attachment and enter circulation, and circulating hemocytes can attach to tissues and become sessile (Babcock et al., 2008; King and Hillyer, 2012; Markus et al., 2009; Sigle and Hillyer, 2016). From a functional perspective, most insects have several sub-populations of hemocytes that are morphologically and functionally distinct, but the nomenclature for these cell populations is not normalized across Insecta. For example, lepidopteran larvae contain four distinct types of hemocytes: plasmatocytes that are involved in capsule formation, granulocytes that drive phagocytosis, oenocytoids that produce enzymes involved in the melanization cascade (e.g., phenoloxidase), and spherule cells whose immune function remains unclear (Lavine and Strand, 2002; Strand, 2008). Mosquitoes, on the other hand, have phagocytic granulocytes, phenoloxidase-producing oenocytoids, and prohemocytes (Castillo et al., 2006; Hillyer et al., 2003a; Hillyer and Strand, 2014). As a final example, the fruit fly, *Drosophila melanogaster*, has phagocytic plasmatocytes, phenoloxidase-producing crystal cells, and encapsulating lamellocytes (Honti et al., 2014).

The fat body, the midgut and the salivary glands are also important drivers of the insect immune response. The fat body, which is composed of loosely associated cells that are rich in lipids and glycogen, lines the integument of the hemocoel (Costa-Leonardo et al., 2013; Larsen, 1976; Martins et al., 2011). Besides functioning in energy storage and the synthesis of the vitellogenin precursors required for the production of eggs, the fat body produces and secretes antimicrobial peptides with lytic activity as well as additional components of the humoral immune response (Aggarwal and Silverman, 2008; Hillyer, 2010; Wang et al., 2014a). The midgut, an organ that primarily functions in digestion and absorption of nutrients, extends almost the entire length of the abdomen and produces nitric oxide synthase and other lytic factors that kill pathogens that are either in the lumen of the gut or are attempting to enter the hemocoel by traversing this epithelium (Buchon et al., 2009; Gupta et al., 2009; Lehane et al., 1997; Lim et al., 2005; Luckhart et al., 1998). The salivary glands, an organ primarily involved in the initial stages of feeding, are usually located in the anterior of the thorax and produce factors that impact the viability of infecting microorganisms (Ferrandon et al., 1998; Pinto et al., 2008).

These immune tissues, although physically and morphologically independent, interact during the course of an infection. For example, factors produced by the fat body induce immune activity in hemocytes (Schmid et al., 2014). Conversely, hemocyte-produced factors are required for immune activity in the fat body (Brennan et al., 2007; Shia et al., 2009). Humoral immune factors that are produced by hemocytes are also transported to the gut where they exert their lytic and melanizing activity (Fraiture et al., 2009), and midgut-produced factors can activate immune activity in the fat body (Wu et al., 2012).

3. Pathogen recognition

The initiation of an immune response requires that the insect recognize an invading agent. During an infection, such recognition usually occurs when pathogen-associated molecular patterns (PAMPs, also known as microbe-associated molecular patterns or MAMPs) bind to host-derived pattern recognition receptors (PPRs). PRRs recognize conserved motifs that are present in microbes but are absent in insects. Examples of PAMPs are bacterial peptidoglycans and fungal β-1,3 glucans.

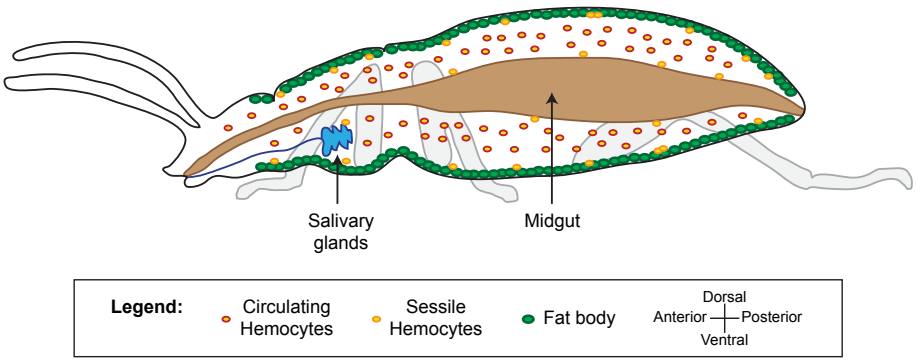

Fig. 1. Anatomy of the insect immune system. The insect body cavity, called the hemocoel, is a fluid and dynamic space that houses tissues with immune activity. The primary immune cells are the hemocytes. Hemocytes are found in circulation (circulating hemocytes) and attached to tissues (sessile hemocytes), where they phagocytose, encapsulate and nodulate pathogens, and produce humoral immune factors. The fat body, the midgut, the salivary glands, and other tissues produce numerous humoral immune factors with, among other things, lytic and melanizing activity. For a description of how hemolymph circulation impacts immune responses, and the activity of periostial hemocytes, see Fig. 7.