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MINIREVIEW

Death for survival: what do we know about innate immunity and cell death in insects?

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Abstract

Insects are the most diverse and prolific animal group on Earth, and as such, important lessons can be taken from the elements that contribute to their evolutionary success. This review examines insect immunity and how insects combat infection with the pathogens they encounter: bacteria, viruses, fungi and parasites. Structural barriers, cellular and humoral responses and cell death all respond to specific immunological threats and contribute to the robust repertoire of immune strategies employed by insects. We discuss the strategies used by insects to combat pathogen infection and focus on what is currently known about cell death and its role in insect immunity.

Key Words: insect immunity; innate immunity; immune signalling; cell death; apoptosis; autophagy

Introduction

Over their 400 million years of evolution, insects have become the most diverse and prolific animal group to inhabit land. There are more than one million species of insects identified to date, with the true number of species estimated at 5 million (Grimaldi and Engel, 2005). Insects play important roles in nearly all areas of terrestrial and freshwater ecosystems; they are pollinators, aid in decomposition and redistribution of nutrients, are custodians of forests and soil, and are vectors of human, animal and plant disease.

The incredible diversity and evolutionary success of insects is due, in no small part, to robust defences against infection. The niches insects occupy bring them into intimate contact with pathogens; bacteria, fungi, viruses and parasites. Of particular importance, insects transmit some of the most devastating human and livestock diseases. In 2008, worldwide cases of malaria, transmitted by *Anopheles* mosquitoes, were estimated at 190-311 million with over one million deaths. Dengue fever transmitted by *Aedes* mosquitoes, Leishmaniasis transmitted by phlebotomine sandflies, Onchocercaiasis, African trypanosomiasis, and Chagas' disease all take an incredible toll on human health, economic growth and development on a global level each year (Dias *et al.*, 2002; Gubler, 2002; Kabayo, 2002; Frick and Foster, 2003; Desjeux, 2004; Sharma *et al.*, 2006). How insects combat infections and more importantly, survive the pathogen loads required to transmit human disease, has been the focus of much research. Here we review insect survival strategies from structural barriers to infection, to cellular and humoral responses, and focus on cell death and its role in insect immunity.

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Insect Immunity: a general overview

Insects owe much of their success to a potent immune response that effectively combats a broad range of pathogens and plays a key role in mediating the interactions between pathogen and insect host. In insects, the first line of defence consists of structural barriers: the exoskeleton or cuticle, the peritrophic matrix, midgut epithelium and the chitinous lining of the trachea (Lowenberger, 2001; Hoffmann, 2003). Once past these barriers, pathogens face a diverse repertoire of immune defences; cellular responses such as hemocyte-mediated responses (*i.e.*, phagocytosis, melanization, and encapsulation) and humoral responses that include the production of reactive oxygen species and antimicrobial peptides (AMPs) (Naitza and Ligoxygakis, 2004; Tanji and Ip, 2005; Ferrandon *et al.*, 2007). Cellular and humoral immune responses are triggered once a pathogen has been identified as non-self. In insect vectors this

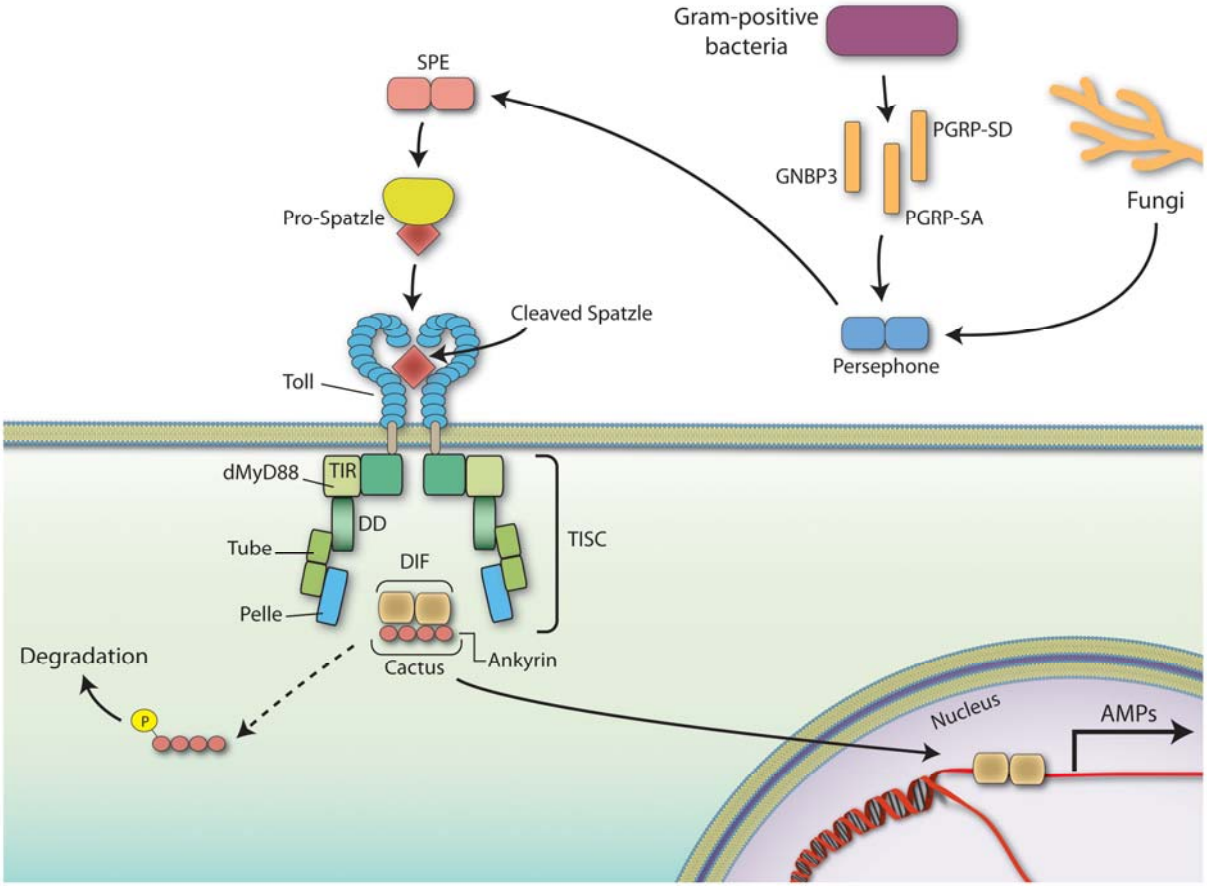

Fig. 1 The Toll pathway in *D. melanogaster*. The Toll pathway is activated by fungi and Gram-positive bacteria. Toll signaling is activated by the binding of a processed form of the cytokine Spätzle to the Toll receptor. During infection, soluble receptors (PGRP-SA, PGRP-SD, GNBP1, GNBP3) recognize pathogens and trigger proteolytic cascades involving the serine proteases Persephone and SPE (Spätzle processing enzyme). SPE ultimately targets and cleaves Spätzle. Once bound to the Toll receptor, signalling through Toll requires the formation of the TISC complex (Toll induced signalling complex), composed of three death domain containing proteins, MyD88, Tube and Pelle. By a mechanism still unclear, Cactus [a homologue of the mammalian inhibitor of NF-κB (IκB)], is phosphorylated, poly-ubiquitinated and targeted for degradation. Once released, the Rel transcription factor Dif (Dorsal-related immunity factor) enters the nucleus and binds to NF-κB response elements and induces the transcription of genes encoding antimicrobial peptides.

process is mediated by pattern recognition receptors (PRRs) that bind to structures on the surface of pathogens referred to as pathogen associated molecular patterns (PAMPs).

Several families of proteins are believed to be involved in distinguishing different classes of pathogens and stimulating appropriate immune responses. PRRs recognize a limited but conserved set of PAMPs that include bacterial lipopolysaccharides (LPS), peptidoglycan (PGNs), lipotechoic acids (LTA), mannans, and fungal 1,3-glucans (Royet, 2004a, b; Warr *et al.*, 2008). Once PRRs recognize invaders as non-self, they trigger a variety of defence reactions that either mediate pathogen killing directly through phagocytosis and melanization, or indirectly through the activation of proteolytic cascades and signaling pathways that control the expression of immune effector genes. The most rapid immune responses include the direct phagocytosis of microbial pathogens by hemocytes and the activation of the prophenoloxidase activating cascade that leads to the melanization of invading microorganisms (Lowenberger, 2001). Shortly thereafter, effector molecules such as anti-microbial peptides (AMPs) can be detected in the hemolymph. AMPs are a powerful component of the insect immune response; they are rapidly produced and have activity specific to a given class of pathogens. AMPs are synthesized by the fatbody, epithelia in the midgut or trachea, and hemocytes, and are regulated principally by the Toll and Immune Deficiency (IMD) signalling pathways (Khush *et al.*, 2001; Lowenberger, 2001; Ferrandon *et al.*, 2007).

Immune signaling pathways

In general, innate immunity in insects is governed by signaling pathways that trigger AMP production, phagocytosis, encapsulation and

melanization. *Drosophila melanogaster* has traditionally been the main model organism for studies of invertebrate immunity and knowledge gained from these studies has been applied to other invertebrate systems. In *D. melanogaster*, the production of AMPs is regulated either by the Toll or IMD (immunodeficient mutant) pathways (Silverman and Maniatis, 2001; Hoffmann, 2003; Ferrandon *et al.*, 2004, Lemaitre and Hoffmann, 2007). The Toll pathway regulates the responses to both fungi and Gram-positive bacteria, and initiates the melanization cascade and blood cell proliferation (Fig. 1). Activation of the Toll pathway during septic challenge triggers two *D. melanogaster* NF-κB transcription factor homologs, Dorsal and Dif, and the production of two AMPs, Drosomycin and Metchnikowin (Ferrandon *et al.*, 2007, Lemaitre and Hoffmann, 2007). The IMD pathway is activated primarily by Gram-negative bacteria, as well as a few Gram-positive bacteria and fungi, and results in the induced expression of the AMPs, Cecropin, Drosocin, Defensin and Diptericin (Kaneko and Silverman, 2005, Tanji and Ip, 2005) (Fig. 2). Some immune-induced genes, such as *diptericin*, are dependent on a single signaling pathway, but others can be induced by both cascades suggesting interactions between the Toll and IMD pathways during septic challenge.

The signaling cascades that control Toll and IMD-mediated transcription of immune effector genes are important mediators of immune responses against extracellular pathogens, such as bacteria and *Plasmodium* (Meister *et al.*, 2005; Vlachou *et al.*, 2005; Dong *et al.*, 2006) that exist transiently in insect hemolymph. The ability of these pathways or others to mediate infections caused by intracellular pathogens, such as viruses are less clear.

There is mounting evidence that *D. melanogaster* and mosquitoes activate signaling pathways that lead to the expression of a distinct set of genes when faced with viral infection. Genome-wide screens of *D. melanogaster* infected with *Drosophila* C virus (DCV) revealed approximately 150 genes are induced 24 - 48 h after infection and many of these genes are not induced by challenge with bacteria or fungi (Sabatier *et al.*, 2003). This suggests that flies, and likely mosquitoes, use distinct strategies to counteract intracellular pathogens. The Toll, IMD and Janus kinase-STAT (Jak-STAT) signaling pathways have all been implicated in response to virus infection (Harrison *et al.*, 1998; Hu *et al.*, 2000; Agaisse and Perrimon, 2004; Gilbert *et al.*, 2005; Smartt *et al.*, 2009; Ramirez and Dimopoulos, 2010; Luplertlop *et al*., 2011). While the Jak-STAT pathway appears to be involved in antiviral immunity, Jak-STAT responsive genes are not induced in immune responsive tissues or virus-infected tissues, only in uninfected and non-immune tissues (Dostert *et al.*, 2005). This suggests that the Jak-STAT pathway may represent a cytokine-mediated signaling cascade involved in communicating with non-infected cells (Beutler *et al.*, 2007). The induction of Toll and Jak-STAT signalling pathways have also been reported in mosquitoes following infection with Japanese encephalitis virus and in shrimp infected with white spot syndrome virus (Lin *et al.*, 2004; Thoetkiattikul *et al.*, 2005; Georgel *et al.*, 2007).

One of the more established antiviral mechanisms in insects is RNA interference (RNAi). RNAi is an ancient form of defence activated by dsRNA to trigger host-mediated degradation of viral RNA. Components of the RNAi pathway are conserved in fruit flies and mosquitoes where they have been reported to play a role in antiviral immunity. In *D. melanogaster*, RNAi deficient mutants are highly susceptible to several viruses including DCV, Cricket Paralysis Virus, Flock house virus, Drosophila X Virus (DXV) and Sindbis virus (Galiana-Arnoux *et al.*, 2006; van Rij *et al.*, 2006; Wang *et al.*, 2006). Similar trends have been observed in mosquito C6/36 cell lines and adult *Aedes aegypti* challenged with Dengue virus specific dsRNA prior to infection with Dengue virus (Gaines *et al.*, 1996; Adelman *et al.*, 2002; Caplen *et al.*, 2002; Olson *et al.*, 2002; Sanchez-Vargas *et al.*, 2004). The same pattern was demonstrated for alphavirus infections in *Anopheles gambiae*, and Keene *et al.* (2004) showed that O'nyong nyong virus (ONNV) replication was significantly reduced when the virus was co-injected with dsRNA derived from the viral genome (Keene *et al.*, 2004).

Cell death and the immune response

Cell death is a key process that tailors host-pathogen interactions. Intracellular infections, particularly viral infections, provoke a dynamic interaction between the host immune system and pathogen immune avoidance strategies to create a suitable environment for pathogen replication. It is difficult for a pathogen to infect a cell without activating any number of immune pathways, including cell death pathways (Clouston and Kerr, 1985; Everett and McFadden, 1999).

Cell death plays an important role in all multicellular animals; it is involved in the formation and deletion of structures during development, maintenance of tissue homeostasis, and eliminating abnormal or damaged cells. Three forms of cell death have been defined based on the morphology of dying cells. Type I programmed cell death, more commonly known as apoptosis, is an intrinsic form of cell death defined by nuclear condensation and fragmentation, DNA laddering (200 bp fragments), blebbing or rounding of the cell, and the externalization of phosphatidylserine (Thornberry and Lazebnik, 1998; Aravind *et al.*, 2001; Kornbluth and White, 2005). Cells dying by apoptosis often fragment into membrane-bound apoptotic bodies that are readily phagocytosed and digested by macrophages or by neighbouring cells without generating an inflammatory response. Apoptotic cell death requires energy in the form of ATP (Edinger and Thompson, 2004). Type II, or autophagic cell death, is a process that denotes self-cannibalization through a lysosomal degradation pathway (Edinger and Thompson, 2004; Levine and Deretic, 2007; Levine and Kroemer, 2008). Cells undergoing autophagic cell death are characterized by the presence of autophagic vacuoles (autophagosomes) and auto-phagolysosomes. Autophagic cell death occurs primarily

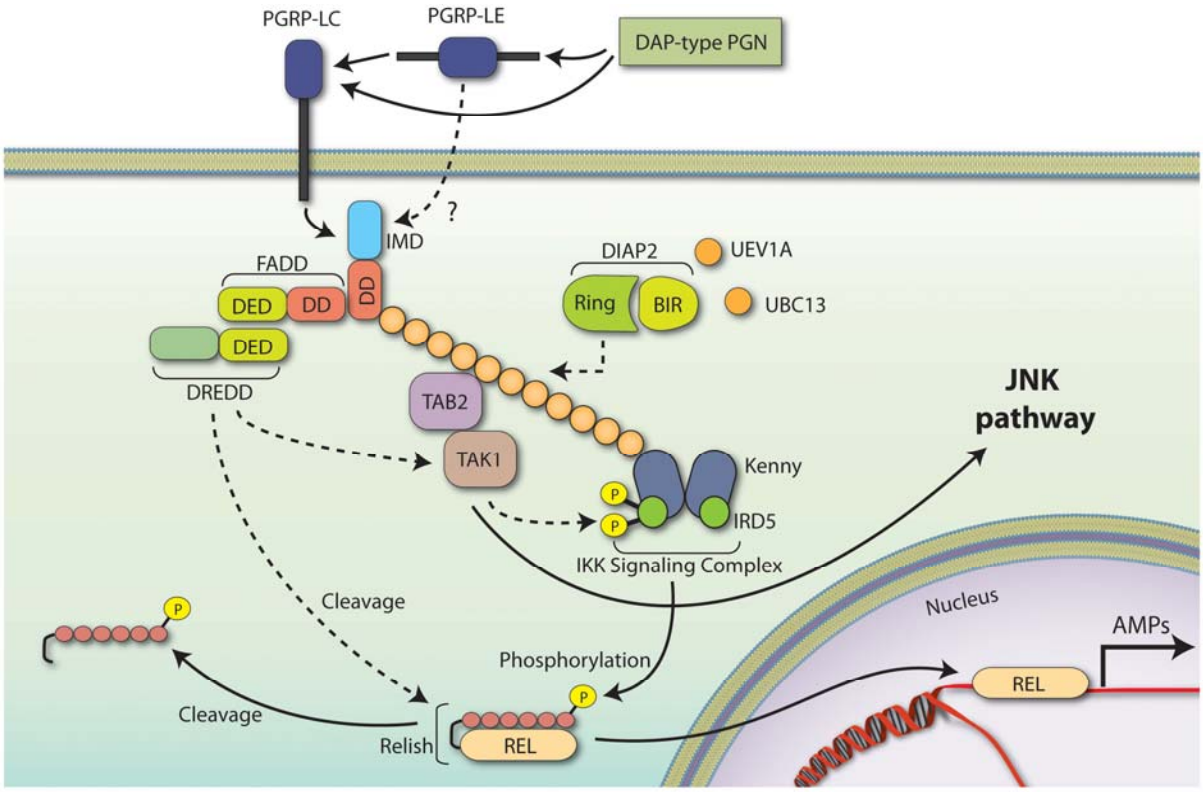

Fig. 2 The IMD pathway in *D. melanogaster*. The IMD pathway is activated in response to Gram-negative bacteria. Peptidoglycan recognition receptors-LC and –LE (PGRP-LC, PGRP-LE) recognize DAP-type peptidoglycans (DAP-type PGN) associated with Gram-negative bacteria and activate IMD through the recruitment of protein, IMD. IMD interacts with the adaptor, dFADD, which itself interacts with the caspase Dredd. Dredd, by an unknown mechanism, is involved in the cleavage of Relish after phosphorylation by the IKK complex, which itself is thought to be activated by dTAK1 and the adaptor dTAB2 in an IMD- and dFADD-dependent manner. The RING domain of DIAP2 may be involved in the activation of dTAB2. Following Relish cleavage, the Rel domain translocates to the nucleus to induce the transcription of several genes, including those encoding antimicrobial peptides. Dashed lines represent mechanisms of unknown function.

when the developmental program requires massive cell elimination but also may be used to degrade intracellular bacteria and viruses (Levine and Kroemer, 2008). This process occurs independent of phagocytes. Lastly, type III cell death, often referred to as necrosis, involves the swelling of organelles, lysosome-independent vacuolation of the cytoplasm, and breakdown of the plasma membranes (Fiers *et al.*, 1999; Proskuryakov *et al.*, 2003; Edinger and Thompson, 2004).

Apoptosis as an immune response

Apoptosis is a process inherent to all eukaryotic cells and has been highly conserved through evolution (Raff, 1998; Hengartner, 2000; Kaufmann and Hengartner, 2001). Apoptosis is an active process that is initiated by the cell and governed by gene activities that either induce or inhibit cell death. Pro-death stimuli are either generated by neighbouring cells or originate within doomed cells. In all cases, a death stimulus will activate a cellular sensor that transmits a signal to downstream proteins, which initiate the appropriate response. The end point of the cell-death signaling cascade is the activation of an evolutionarily conserved family of cysteine proteases called caspases (Thornberry and Lazebnik, 1998; Aravind *et al.*, 2001; Kornbluth and White, 2005). In mammals, apoptosis is triggered by death signals that result in the activation of initiator caspases and the subsequent activation of downstream effector caspases. Activity of pro- and anti-apoptotic members of the Bcl-2 family of proteins govern caspase activation and the apoptotic process. Once initiated, caspase activity is dampened by several mechanisms, principally by the IAP family of proteins. Although many components of the apoptotic pathways are conserved in insects, the pathways themselves differ slightly between species. Much of our current understanding about apoptosis in insects comes from studies in fruit flies. In *D. melanogaster* there are three initiator caspases (Dronc, Dredd and Strica) and four effector caspases (Drice, DCP-1, Damm and Decay). Among these, Dronc and Drice are the core capases involved in apoptosis with DCP-1 playing a minor role only evident in certain cell types in the absence of Drice. In contrast to

mammals, where caspase activation serves as the main point of control, cells in *D. melanogaster* experience chronic activation of the apical caspase Dronc (Fig. 3). Cells survive because they express the Inhibitor of Apoptosis Protein1, DIAP1 (Hay and Guo, 2006; Steller, 2008). DIAP1 suppresses Dronc activity, and therefore the activity of downstream caspases activated by Dronc. The expression of the pro-death RGH proteins (Reaper, Grim, Hid, Sickle, Jafrac2) disrupt DIAP1-caspase interactions, resulting in the activation of the caspase cascade (Orme and Meier, 2009). Less is known about cell death signalling in other insects, though a significant amount of research has recently been focused on apoptosis in mosquitoes.

Fruit flies and mosquitoes are separated by approximately 250 million years of evolution and have been exposed to very different evolutionary pressures. The genome projects of *A. gambiae* and *A. aegypti* have revealed that mosquitoes have expanded families of caspases and IAPs which may be due in part to exposure to pathogens ingested during blood feeding. Although mosquitoes are a relatively new model with respect to cell death, studies indicate that signalling pathways appear to be conserved when compared to *D. melanogaster* (Bryant *et al.*, 2008; Cooper *et al.*, 2007a, b, 2009; Liu and Clem, 2011; Wang and Clem, 2011). It should be noted that an expansion of caspases has also been noted in other *Drosophila sp*., although roles for these additional caspases have not yet been identified (Bryant *et al.*, 2010).

As insects lack an adaptive immune response, one would predict apoptosis to play a central role in antiviral and anti-parasite immunity. Induction of cell death is a powerful immune response because it has the potential to severely limit pathogen production and to reduce or eliminate the spread of pathogens within the host. The importance of apoptosis as an immune defence mechanism is borne out by the large number of animal viruses that encode proteins specifically to interrupt cell death pathways. To date, more than a dozen viral genomes have been shown to encode proteins that modulate apoptosis (Teodoro and Branton, 1997; Benedict *et al.*, 2002). Viruses use various strategies to impede apoptosis; in some cases targeting a single point of the process is sufficient to affect the onset of the cell death program. Some viruses interfere with cellular sensors before they trigger apoptotic cell death, some encode gene products that inhibit the sensors of cell cycle manipulations and others target double stranded RNA activated protein kinase (PKR) (Katze, 1995; Teodoro and Branton, 1997; Barry and McFadden, 1998; Roulston *et al.*, 1999; Barber, 2001; Benedict *et al.*, 2002; Hay and Kannourakis, 2002).

Viruses may also encode gene products that directly block the apoptotic signaling cascade or prevent the activation of caspases. Several DNA viruses, including Adenoviruses and Epstein-Barr virus (EBV) encode gene products functionally analogous to the anti-apoptotic protein, Bcl-2. The cytokine response modifier A (CrmA) encoded by the cowpox viruses, p35 and IAP proteins encoded

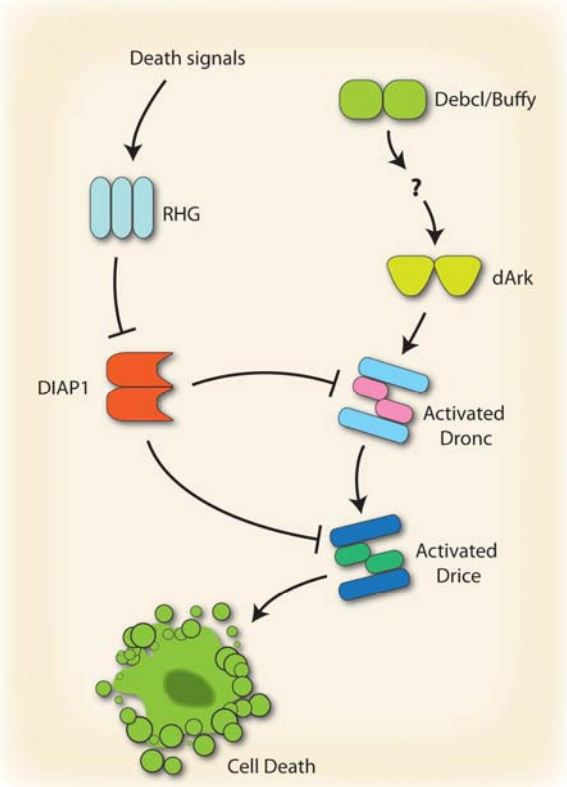

Fig. 3 Schematic of the cell death pathway in *D. melanogaster*. Both organisms provide a different paradigm for the induction of apoptosis through caspase activation. In *C. elegans*, the BH3-only protein, EGL-1, is upregulated in cells destined to die. EGL-1 is a pro-apoptotic protein that binds to and inactivates the inhibitor protein, CED-9. The release of CED-9 results in the activation of CED-4, which in turn activates the sole death caspase, CED-3. In *D. melanogaster*, the primary apoptotic caspase is Dronc, a caspase-9 homolog with an N-terminal CARD domain for interaction with the Apaf-1 homolog dARK. Dronc activation is primarily regulated by the RHG proteins (Reaper, Hid, Grim, Sickle, and Jafrac2). Together, these proteins promote cell death by binding to the inhibitor of apoptosis protein DIAP1, disrupting anti-caspase activity of DIAP1. Once activated, Dronc will cleave and activate the effector caspases Drice and Dcp-1. No firm role in apoptosis has been established for the *D. melanogaster* Bcl-2 proteins (Buffy and Debcl).

by the baculoviruses, and viral homologues of the cellular apoptosis inhibitor known as cFLIPS all regulate caspase activity (Birnbaum *et al.*, 1994; Clem and Miller, 1994; Thome *et al.*, 1997; Chaudry *et al.*, 1999). Smaller viruses, such as the RNA viruses, have developed alternative strategies like inducing the expression of endogenous anti-apoptotic genes, including host Bcl-2 and IAP genes (Liao *et al.*, 1997; Teodoro and Branton, 1997; Liao *et al.*, 1998; Hay and Kannourakis, 2002). In some cases, Bcl-2 expression is able to modulate the

effects of viral infection and over-expression of Bcl-2 allowed for the establishment of persistent infection (Liao *et al.*, 1998; Su *et al.*, 2001). Rapid replication and dissemination have also been reported as tactics used by RNA viruses to avoid apoptotic cell death (Koyama, 1995; Kurokawa *et al.*, 1999).

While apoptosis is a commonly reported and extensively studied immune response in mammals, comparatively less is known about apoptosis and immunity in insects. Studies in *Drosophila* cell lines have shown that Flockhouse virus will induce apoptosis by depletion of DIAP1 through virus-induced inhibition of host protein synthesis in *D. melanogaster* (Yoo *et al.*, 2002; Settles and Friesen, 2008). Because DIAP1 has a short half-life (35-40 min), reductions in protein synthesis would create depletion in the intracellular pool of DIAP1 resulting in apoptosis (Yoo *et al.*, 2002). Apart from a small number of studies in *Drosophila*, much of the evidence collected to date has been observational, showing only associations between infection and apoptosis.

Perhaps the most compelling evidence linking apoptosis to immunity has been observed in mosquitoes. For many years, arthropod-borne viruses, such as dengue virus and yellow fever virus, were believed to cause lytic infections in mammalian cells but persistent infections with only moderate cytopathic effects in mosquito cells. More recently, *in vivo* studies have provided strong evidence that apoptosis may play a role in mediating virus infections in mosquitoes and therefore may impact vector competence. Apoptosis-linked pathologies have been described in *A. aegypti* infected with Semliki Forest virus (Mims *et al.*, 1966), in *A. albopictus* infected with Sindbis virus (Bowers *et al.*, 2003), and in the salivary glands of *Culex pipiens quinquefasciatus* infected with West Nile virus (WNv) (Girard *et al.*, 2005, 2007). Infections with other intracellular pathogens, such as *Plasmodium sp.*, have also been shown to cause pathologies resembling apoptosis in mosquitoes (Hopwood *et al.*, 2001; Al-Olayan *et al.*, 2002; Hurd and Carter, 2004; Hurd *et al.*, 2006). *P. berghei* infection causes apoptosis in midgut cells of *A.. stephensi* (Han *et al.*, 2000) and *A. gambiae* (Vlachou *et al.*, 2004), while *P. gallinaceum* infection is associated with apoptosis in *A. aegypti* (Zieler and Dvorak, 2000). In addition, caspase activity is associated with *Plasmodium* infections in mosquitoes (Han *et al.*, 2000; Zieler and Dvorak, 2000; Abraham *et al.*, 2004; Vlachou *et al.*, 2004).

Although these studies are largely observational, they suggest that apoptosis is associated with vector competence in mosquitoes. Vaidyanathan and Scott (2006) showed extensive cell death in the midgut cells of a lab-derived strain of *C. pipiens pipiens* refractory to infection with WNv upon exposure to the virus (Vaidyanathan and Scott, 2006). Additionally, reduced virus transmission is associated with apoptosis in the salivary glands of *C. pipiens quinquifasciatis* during the later stages of WNv infection. These studies demonstrate that host cells detect virus infection and attempt to clear the infection through induction of cell death. The events that trigger virus-induced apoptosis and the mechanisms that viruses use to evade apoptosis remain largely unknown; however, this field of research holds promise in illuminating new determinants of vector competence in mosquitoes.

The strongest evidence linking apoptosis to insect immunity comes from a family of viruses known as the Baculoviruses. Baculoviruses are large DNA viruses, most often associated with Lepidoptera, that can trigger rapid and wide spread apoptosis in insect cells following replication in host cell cytoplasm, (reviewed in Clarke and Clem, 2003; Clem, 2005, 2007). Consequently, Baculoviruses encode their own apoptotic supressors including viral IAPs and caspase inhibitors (P35 and P49) to block premature cell death and promote virus multiplication (reviewed in Clem, 2005, 2007). IAPs have multiple functions that include binding and inhibiting caspase activity, regulating cell cycle progression, and modulating receptor-mediated cell cycle signal transduction (Deveraux and Reed, 1999, Salvesen and Duckett, 2002). P35 and P49 are pancapase inhibitors that prevent the apoptotic response in cells by binding to the active site of caspases (Lannan *et al.*, 2007). Given that both IAPs and caspase inhibitors function downstream of the events that trigger apoptosis, it is likely that the host cell initiates apoptosis in response to viral infection while the baculovirus inhibits cell death to promote cell survival. Recently, Liu *et al*. (2011) used an *in vivo* system to demonstrate Michelob_X (mx), the mosquito ortholog of pro-apoptotic gene *Drosophila* reaper, is specifically induced in larval *C. nigripalpus* midgut cells following infection with a baculovirus (Liu *et al.*, 2011). In the permissive mosquito *C. quinquefasciatus*, a slow induction of mx failed to induce apoptosis; infected cells eventually underwent necrosis due to high virus loads. In contrast, a rapid induction of mx within 30 min post-infection followed by apoptosis within 2 - 6 h post-infection occurred in the refractory mosquito, *A. aegypti* (Liu *et al.*, 2011). These data suggest a possible role for apoptosis in limiting viral infection. Further research aimed at understanding apoptosis in insects, particularly those that vector disease, will provide a promising platform for new research into host-pathogen interactions.

Autophagy as an immune response

Autophagy, an intrinsic program that degrades cytoplasmic components, has been implicated as a process that responds to many different forms of stress, including microbial infection. Typically, autophagy transpires at basal levels in all cells and serves as the major mechanism in homeostatic functions such as the turnover of damaged proteins and organelles, and is essential for survival, differentiation, and development (Codogno and Meijer, 2005). The autophagy-related genes (*atg*), a family of conserved genes which encode proteins required for various phases of the autophagic pathway, are found in organisms ranging from the simple unicellular *Saccharomyces cerevisiae*, to the multicellular organisms including insects and mammals (Galluzzi *et al.*, 2008). In multicellular animals, conserved signalling pathways also

regulate autophagy. The Mammalian Target of Rapamycin (mTOR) kinase pathway plays a pivotal role as a sensor of cellular energy, growth factor and nutrient levels and will inhibit autophagy when these factors are abundant. When nutrients are limited, mTOR is inactivated and autophagy is induced (reviewed in McPhee and Baehrecke, 2009). Upstream of mTOR, members of the insulin receptor/class I phospho-inositide-3-kinase (PI3K) pathway also repress autophagy in a nutrient-dependent context. Additional signaling pathways such as the tumor repressor PTEN promote autophagy through antagonizing PI3K signalling (McPhee and Baehrecke, 2009). Whereas basal levels of autophagy ensure the physiologic turnover of damaged organelles, the massive accumulation of autophagic vacuoles has been linked to an alternative cell death pathway, type II or autophagic cell death. Whether the autophagic vacuoles associated with type II cell death are the results of failed attempts to adapt to cellular stress or whether they represent a unique non-apoptotic form of cell death is debatable and beyond the scope of this review. Below we discuss what is currently known about autophagy and microbial infections in insects and highlight current limitations in the field.

As with apoptosis, relatively little is known about the role autophagy plays in immunity in insects. Autophagy can be triggered in infected cells where it may act as a host defence mechanism to eliminate a pathogen without eliminating the cell (Kirkegaard *et al.*, 2004; Levine and Deretic, 2007). In mammalian systems, autophagy has been to shown to clear bacteria from the cytoplasm and restrict the spread or replication of several viruses including Herpes simplex virus 1 (HSV-1), Human immunodeficiency virus -1 (HIV-1) and Sindbis virus (Liang *et al.*, 1998; Gutierrez *et al.*, 2004; Andrade *et al.*, 2006; Ling *et al.*, 2006; Singh *et al.*, 2006; Talloczy *et al.*, 2006). Numerous studies have shown that insects contain all the machinery to induce and regulate autophagy; however, autophagy and/or autophagic cell death is almost exclusively observed during developmental periods when massive cell or tissue elimination occurs (Malagoli *et al.*, 2010). Few reports exist describing an immune role for autophagy or autophagic cell death in insects. Two exceptions, a study by Shelly *et al*. (2009) and a study by Cherry *et al*. (2009) have demonstrated that autophagy is observed in *Drosophila* infected with mammalian vesicular stomatitis virus (VSV). Here, induction of autophagy was associated with decreased viral replication and repression of autophagy led to both increased viral replication and pathogenesis in both cell lines and adult flies. Autophagy can be controlled by the well-conserved phosphatidylinositol 3-kinase (PI3K)-Akt-signaling pathway, which normally mediates autophagy in response to nutrient availability. Interestingly, the activation of autophagy in response to VSV infection did not require virus replication, suggesting that cells were sensing the virus itself, perhaps through a PAMP. In contrast to VSV infection, flies depleted of Atg18, a conserved component of the PI3K-Akt-signaling pathway, were not more sensitive to infection with DCV, suggesting that the autophagy-mediated antiviral response is protective against VSV but not DCV infection. Why autophagy is observed with VSV infection but not DCV infection is not known but remains an interesting question. It is important to note that the field of autophagy and autophagic cell death is only now gaining adequate attention. As the field develops and more tools become available, valuable knowledge regarding the role autophagy plays in antiviral or anti-parasite responses in insects should be uncovered. This is of particular interest for vectors of disease as they must maintain tissue integrity and promote cell survival while carrying large pathogen loads.

Concluding remarks

Insects are armed against invading pathogens with a diverse and powerful repertoire of defences. Innate immunity, indeed all immune responses, requires identification of a pathogen as non-self. Insect PRRs and PAMPs engage cellular machinery to eliminate pathogens through phagocytosis, melanization, proteolytic cascades, AMPs, RNAi and the Toll, IMD, Jak-STAT pathways.

Insects and the pathogens they transmit evolve in concert; an arms race to outwit the other's defence and invasion strategies in turn. Insects are reliant on innate immunity to overcome infection and in the absence of acquired immunity; cell death can be a powerful mechanism for the elimination of pathogens that evade the innate immune response. As the death of an infected cell is often concomitant with the death of the infecting agent, cell death can promote efficient pathogen clearance. The abundance of anti-apoptotic proteins encoded by animal viruses attests to the importance of apoptosis as a measure of combating infections. Although cell death is a comparatively new field in non-drosophilid insects, numerous studies now link cell death to immunity in insects. As new information is gathered on cell death signalling in insects and more tools become available to study the various forms of cell death, we can begin to ask questions about the signalling events that trigger cell death and strategies used by pathogens to counteract this response. The mosquito model, in particular, provides the opportunity for a comparative study of cell death and the regulation of specific immune processes in insects in which intracellular pathogens infect host tissues and multiply before being transmitted to humans. New ways of combating arboviral transmission must be developed and understanding how insects interact with the pathogens they carry is an important step.

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