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LETTER TO EDITOR

Insect anti-viral immunity: roles of prostaglandins and other eicosanoids

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To the Editor

Insect/microbe relationships are very complex, with an array of signaling systems acting in surveillance, detection and responses to the presence of beneficial, neutral and harmful microbial species within insects. The emerging view indicates prostaglandins (PGs) and other eicosanoids are responsible for essential signaling in activating and coordinating insect innate immune reactions to infections, including viral infections. The structures, biosynthesis and history of eicosanoids are detailed elsewhere (Stanley and Kim, 2014).

Reports of PG actions in insects emerged in the 1970s. Loher (1979) reported that PGE_2 releases egg-laying behavior in a cricket species. Dalton (1977) found that PGE_1 modulates salivary gland fluid secretion in a blowfly. Stanley and his colleagues discovered that eicosanoids signal insect cellular immune reactions to bacterial infections (Stanley-Samuelson *et al*., 1991). This finding stimulated research into the biochemistry of PG production and PG signaling mechanisms. In this Letter, we outline PG actions in insect immunity and then draw attention to recent reports on PG actions in anti-viral immunity.

Insect hemocytes make up the immediate defense against microbial infections and parasite invasions. Cellular actions include phagocytosis, encapsulation and nodulation. Phagocytosis and formation of melanotic nodules is responsible for clearing the majority of infecting microbes from hemolymph circulation. Remaining microbes are cleared by production of antimicrobial peptides (Haine *et al.,* 2008). PGs mediate microaggregation and nodulation reactions (Miller *et al.,* 1994). These are complex cellular processes. For example, 184 gene products act in phagocytosis in *Drosophila* S2 cells (Stroschein-Stevenson *et al*., 2006). Phagocytosis is mediated by PGs in hemocytes prepared from wax moths, *Galleria mellonella* (Mandato *et al*., 1997) and *Spodoptera exigua* (Shrestha and Kim, 2007), as well as from the blood sucking bug, *Rhodnius prolixus* (Figueiredo *et al*., 2008). Hemocytes migrate to the sites of wounds and infection, and hemocyte migration also is signaled by eicosanoids (Merchant *et al*., 2008).

The first step in eicosanoid biosynthesis is the hydrolysis of AA from membrane-associated phospholipids (PLs) by action of a phospholipase A_2 (PLA_2). Shrestha *et al.* (2010) showed that bacterial challenge increased PLA_2 activity in plasma and, separately, in hemocyte/fat body preparations from flour beetle, *Tribolium castaneum*, larvae. We identified and cloned five *T. castaneum* genes encoding secretory PLA_2s ($sPLA_2$s), $TcsPLA_2$A through E, and showed that all five recombinant $TcsPLA_2$s have PLA_2 activity. dsRNA constructs specific to each of the five genes silenced expression of four genes from 24 to 72 h after treatment, 48 to 72 h for $TcsPLA_2$D. In separate experiments, silencing each of these genes, except $TcsPLA_2$C, suppressed flour beetle immunity, determined as nodulation following a standard bacterial challenge. Hence, these four genes are directly involved in eicosanoid signaling.

The insect cytokine, plasmatocyte spreading peptide (PSP) stimulates spreading in subpopulations of lepidopteran plasmatocytes (Clark *et al.,* 1997). Kim *et al.* (2008) found that PSP also influenced hemocyte spreading in *S. exigua*. Work in Kim's laboratory later showed that PSP mediates hemocyte spreading via eicosanoid signaling (Srikanth *et al*., 2011). Later work revealed the actions of PSP and PGE_2 in hemocyte spreading are coordinated via the small G protein, Rac1 (Park *et al.,* 2013). Expression of *SeRac1* increased by about 8-fold at 2 h post infection (PI) and a dsRNA construct silenced *SeRac1* expression for at least 96 h following infection. Silencing *SeRac1* negated PSP-stimulated hemocyte spreading, which was rescued by PGE_2 treatments. This clarified some of the intracellular cross-talk between PSP and PG signaling.

Phenoloxidase (PO) acts in several aspects of insect immunity, including melanization of encapsulated parasitoid eggs and newly-formed nodules. The *S. exigua* PO is biosynthesized in oenocytoids as the zymogen prophenoloxidase

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(PPO). The PPO is released from oenocytoids into hemolymph circulation by cell lysis. Shrestha and Kim (2008) showed that eicosanoids mediate the cell lysis. They found that treating hemocytes with pharmaceutical inhibitors of eicosanoid biosynthesis blocked the cell lysis, which was reversed by AA treatments. They generated more insight into eicosanoid actions, showing eicosanoids mediate the release of PPO via protein kinase C (Shrestha and Kim, 2009). PGs exert their actions in mammalian cells via cell surface G protein coupled receptors (GPCRs; Bos *et al.,* 2004). Oenocytoids express a GPCR that mediates PGE_2-stimulated oenocytoid cell lysis (Shrestha *et al*., 2011). This is the first demonstration of the mechanism of a PG action in insect biology, from which we infer that some PGs act via cell surface GPCRs.

Drawing on the biomedical background, PGs are generated via a cyclooxygenase (COX), an enzyme with two separate active sites. The first site converts AA to the hydroperoxyendoperoxide, PGG_2, which is reduced by the peroxidase site to PGH_2. PGH_2 is a substrate for several enzymes that convert it into various PGs in a cell-specific manner. On the idea that insects also express COXs, PG biosynthesis has been recorded in several insect tissues using enzyme preparations similar to protocols appropriate to mammalian COXs. For one example, a fat body preparation from the tobacco hornworm, *Manduca sexta*, produced PGs from radioactive AA (Stanley-Samuelson and Ogg, 1994). Insect genomic data bases facilitated searches for insect genes encoding COX proteins, which uniformly indicate insects do not have such genes, although two crustaceans do (Varvas *et al.,* 2009). This situation prompts the question of how to explain the presence and actions of PGs in insects? Tootle *et al.* (2011) showed that PG signaling is necessary for the correct temporal expression of genes encoding eggshell proteins. More to the point, they showed that a peroxinectin (Pxt) type peroxidase (POX) is responsible for synthesizing the PGs. Park *et al.* (2014) investigated the idea that a Pxt type POX acts in *S. exigua* immunity. The authors identified ten genes encoding peroxidases from *S. exigua* transcriptomes (*SePOX-A - SePOX-J*) and designed dsRNA constructs to individually suppress expression of each of them. Each of the 10 genes was partially silenced by injecting their cognate dsRNAs into experimental larvae. Silencing each of these genes showed that two of the ten POX genes, *SePOX-F* and *-H* act in hemocyte defense reactions because hemocyte spreading and nodule formation were inhibited in insects injected with dsPOX-F and dsPOX-H, but not in insects injected with the other 8 dsRNA constructs. The influence of dsPOX-F and -H was reversed by co-injections with PGE_2, from which it was concluded that *SePOX-F* and *-H* are Pxt-like genes responsible for PG biosynthesis in *S. exigua*. More broadly, Pxt genes may be responsible for producing PGs in most, if not all, insect species.

Insects express several mechanisms to protect themselves from viral infections. One of the most important mechanisms is caspase-mediated apoptosis and sloughing of virus-infected midgut epithelial cells (O'Neill *et al*., 2015). Constitutive plasma PO may act as an anti-viral defense in lepidopteran larvae (Popham *et al.,* 2004). Washburn *et al.* (1996) recorded hemocytic encapsulation of virus-infected cells in *H. zea* larvae. We have less information on eicosanoid signaling in insect anti-viral immunity, reviewed just below.

Treating gypsy moth larvae, *Lymantria dispar*, with various inhibitors of eicosanoid biosynthesis increased larval susceptibility to its nucleopolyhedrovirus, LdMNPV, from which it was inferred eicosanoids act in insect responses to baculovirus infection (Stanley and Shapiro, 2007). The work was expanded to other lepidopterans, including the beet armyworm, *S. exigua*, the corn earworm, *Heliothis zea* and the fall armyworm, *S. frugiperda* (Stanley and Shapiro, 2009). Again, larvae were treated with a selected inhibitor and then challenged with their respective NPV, SeMNPV, HzSNPV and SfMNPV. At least one of the inhibitors led to increased larval susceptibility to their NPV and the influence of one inhibitor, indomethacin, was expressed in a dose-related manner. The overall inference was that eicosanoids act in insect defenses against NPVs, however, these papers did not provide insight about specific eicosanoid-mediated defense mechanisms.

Insects clear some viral infections from circulation via nodule formation. Using larvae of the greater waxmoth, *G. mellonella*, Büyükgüzel *et al.* (2007), tested the hypothesis that eicosanoids mediate nodulation reactions to a vertebrate virus, bovine herpes simplex virus-1 (BHSV-1). They reported that viral infection increased nodulation in a near linear manner, from about 15 nodules/larva in controls to nearly 200 nodules/larva in experimental larvae injected with the highest viral challenge. Nodulation increased with time PI to a high at 4 h. Injecting the COX inhibitor, indomethacin, into experimental larvae followed by a virus challenge led to sharply decreased nodulation reactions. This work also showed that orally administered indomethacin reduced nodulation reactions to viral infection. In a similar manner, Durmuş *et al.* (2008) found that challenging larvae of the waxmoth parasitoid, *Pimpla turioinellae*, with BHSV-1, also induced nodulation, increasing from about 15 nodules/larva in controls to >50 nodules/larva in infected larvae. Treating parasitoid larvae with one of three pharmaceuticals, indomethacin, the lipoxygenase inhibitor, esculetin, or the glucocorticoid, dexamethasone, inhibited BHSV-1-induced nodule formation. The inhibition was reversed by treating experimental larvae with AA and the authors drew the reasonable conclusion that eicosanoids mediate nodulation reactions to viral infection in the wasp larvae.

Eicosanoids influence expression of host defense genes in the silk-producing insect, *Antheraea pernyi*. Zhang *et al*. (2015a) showed that bacterial, fungal and NPV infections led to increased expression of the *A. pernyi* small heat shock protein, Ap-sHSP21.4, in midgut, hemocytes and fat body of infected larvae. The expression increases occurred in separate temporal patterns, at 3 h PI in fat body, at 24 h PI in midgut and at 12 h PI in hemocytes. Injected siRNA constructs silenced *Ap-sHSP21.4*

expression following NPV challenge and expression of some immunity genes, particularly genes encoding defensin, Toll1 and lysozyme after NPV challenge. The influence of NPV infection on *Ap-sHSP21.4* expression was strongly inhibited by treating experimental larvae with pharmaceutical inhibitors of eicosanoid biosynthesis; treating larvae with the eicosanoid biosynthesis precursor, AA, induced *Ap-sHSP21.4* expression. The authors inferred that *Ap-sHSP21.4* acts in host defense against microbial infections and that eicosanoids mediate infection-related *Ap-sHSP21.4* expression. In a related paper, they also reported eicosanoids act in expression of another small heat shock protein, sHSP20.8 (Zhang *et al.,* 2015b). This work demonstrates a specific molecular mechanism of eicosanoid actions in insect immune reactions to microbial, including viral, infections.

PGs and other eicosanoids are central operators in insect immune signaling and in cross-talk with other signal systems, including an insect cytokine. The identification of several genes involved in eicosanoid signaling creates a sound basis for continued fundamental research into these systems, with the expectation of practical applied outcomes (Stanley and Kim, 2014).

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