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EDITORIAL

Thioester-containing proteins: At the crossroads of immune effector mechanisms

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Work published in this issue of *Virulence* sheds new light on the function of a phylogenetically conserved protein family, which is shown to modulate immune responses in insects such as the fruitfly *Drosophila melanogaster*. Just like in any immune system, insect innate immune reactions are activated through recognition of microbe- or damage-associated patterns, which indicate deviations from tissue or organismic integrity[2]. Microbial surfaces are targeted through binding of dedicated receptors (pattern recognition receptors, PRRs) to microbial polysaccharides, lipids and proteins. PRRs include Gram-negative-binding proteins (GNBPs), peptidoglycan-recognition proteins (PGRPs), scavenger receptors (SRPs) and thioester-containing proteins (TEPs).[2] TEPs make up a family of proteins that are structurally related to vertebrate complement proteins (such as complement component C3), including an intramolecular β-cysteinyl-gamma-glutamyl thioester bond.[4] Other complement components appear absent from insect genomes raising questions about insect TEPs' function. Initial studies on mosquito- and subsequently *Drosophila* TEPs revealed functional similarities to complement namely their involvement in opsonization of microbes and parasites including *Plasmodium* species.[9] Effector functions identified downstream of TEPs include phagocytosis, melanization (the production of cytotoxic intermediates and ultimately production of melanin) and the formation of lytic complexes that kill *Plasmodium*. The last reaction is functionally equivalent to complement-mediated but involves different proteins. The lytic complex in *Anopheles gambiae* comprises TEP1, and 2 leucine-rich proteins (LRIM1 and APL1C), which are all essential for immunity against *Plasmodium*.[3,6]

The *Drosophila* genome contains 4 genes that encode TEPs (Tep1–4,[8] with a canonical thioester motif, one possible pseudogene (Tep5) and a gene encoding a related protein (macroglobulin complement-related, MCR or TEP6). Except for transcriptome studies, which showed induction of TEPs in different infection regimes and *in vitro* work supporting their opsonizing activity, little was known about *Drosophila* TEPs until a few years ago.[14] Functional studies implicated TEPs in protection against some but not other infections.[5] The latter included a natural parasitic nematode (*Heterorhabditis bacteriophora*), which harbors the insect pathogenic bacterium *Photorhabdus luminescens,* and to which TEP3 loss-of-function mutant flies were more susceptible.[1]

In their study in this issue of *Virulence*[13] and related previous work,[12] Eleftherianos and coworkers have used infection with *P. luminescens* and with a human-pathogenic member of the same genus (*Photorhabdus asymbiotica*). Their data provide insight into the regulatory activity of different members of the *Drosophila* TEP family. Their previous work focused on *Drosophila* TEP4, which they showed modulates activation of the 2 major immune signaling pathways (Toll and IMD) both of which respond to *Photorhabdus* infections. Interestingly both pathways were activated in Tep4 loss-of-function mutants, while 2 other pathways implicated in stress responses (Jak/Stat and Jnk) were downregulated. In line with these observations TEP4 also regulated melanization in mosquitos, although *Drosophila* TEP4 appeared to inhibit this reaction.[12] Taken together this indicates that both *Photorhabdus* species manipulate the host response by upregulation of TEP4, which favors stress- at the expense of immune responses.

In their article in this issue of *Virulence*, Shokal et al. expand their studies to include Tep2 and Tep6. They infect loss-of-function mutants for either of these genes with non-pathogenic *Escherichia coli*, as well as with the previously used *Photorhabdus* species. In some combinations that involve infection with either *Photorhabdus*, Tep mutants fare better when checked for survival and

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bacterial counts. However, only Tep6 mutants had a survival advantage after infection with *P. luminescens*. In line with these differences, both the induction pattern of immune and stress pathways and the regulation of melanization differed between the mutants: in several of these assays, Tep2 behaved more similar to Tep4 compared with Tep 6, most likely due to the absence of the thioester motif in Tep6. Functional ablation of hemocytes shows that they contribute substantially to the production of both TEPs after infection. In line with the data from mosquitos, both mutants show a reduction in phagocytic potential and lower levels of one of the major phagocytic receptors (Eater) at different time points post-infection. Taken together these results indicate that both pathogenic *Photorhabdus* species target thioester-containing proteins and their relatives to interfere with the fine-tuning of the hosts immune system. Removing TEPs therefore reduces mortality rates although in the long-term even TEP mutants succumb to the infection. The 2 *Photorhabdus* species behave similar in several assays but there are also differences such as in the effects on Toll signaling and stress-induced pathways at later time points. This may indicate specific adaptations to their respective hosts (insects versus humans). Altogether the specific immune-modulatory effects of *Drosophila* TEPs, make them ideal targets for pathogen derived virulence factors, which may highjack their activity to manipulate host immunity. This in turn is expected to lead to counter-adaptations from the host side leading to an evolutionary arms race. TEPs are therefore expected to undergo evolutionary changes. Indeed evidence for positive selection has been obtained for several TEP family members, whereas other PRRs such as GNBPs appear to be more conserved.[7] Besides evolutionary aspects it will be interesting to study the long-term induction pattern of TEPs after different microbial infection, some of which are already evident from the present study. Also possible changes in the epigenetic markers around TEP genes are worth investigating. It has been proposed that the combinatorial activity of PRRs and their interacting proteins may explain differences in the specificity or even effector mechanism due to qualitative and/or quantitative changes in the concentration of the proteins in the active complex.[11] As mentioned above mosquito leucine-rich proteins (such as LRIM1 and APL1C) are known partners for TEP1, which can alternatively result in melanization, lysis or phagocytosis of the intruder. In combination with functional studies as presented by Shokal et al, characterization of the TEP interactome and the induction kinetics of the genes involved may even provide insight into molecularly less-characterized phenomena such as immune priming[10] where—based on their immune-modulatory activities—TEPs are expected to contribute.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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