Nervous about immunity: neuronal signals control innate immune system

Cheng-Yuan Kao, Ferdinand C O Los & Raffi V Aroian

The molecular mechanisms by which the nervous system influences innate immunity to pathogens remain mysterious. Two new studies show that neuronal products modulate established innate immune signaling pathways operative in the *Caenorhabditis elegans* intestine.

Caenorhabditis elegans is a free-living soil nematode that feeds on live bacteria. Important pathogenic bacteria such as *Pseudomonas aeruginosa* strain PA14, *Staphylococcus aureus* and *Salmonella enterica* can infect and eventually kill *C. elegans*[1]. Given the great power of the genetics and genomics of *C. elegans*, it is not unexpected that this worm has become an influential whole-organism model for studying innate immunity. Several innate immune signaling pathways have been identified in the worm over the past several years, including those involving the p38 mitogen-activated protein kinase (MAPK) ortholog PMK-1 and those involving insulin-mediated signaling through the insulin–insulin-like growth factor 1 receptor ortholog DAF-2 and the transcription factor DAF-16 (ref. 2). Two recent papers[3,4], one in this issue of *Nature Immunology*, break new ground in linking the nervous system of *C. elegans* to innate immunity.

In the context of PA14 infection, one of the main innate immune responses in the worm is a signal-transduction cascade emanating from the Toll–interleukin 1 receptor domain protein to the MAPK kinase kinase NSY-1, to the MAPK kinase SEK-1 and finally to PMK-1; this pathway regulates the expression of many genes encoding host-defense effector molecules in intestinal cells[2,5]. So far, most studies of the innate immunity of *C. elegans* have focused on the intestinal tract, as ingested pathogens must be successfully dealt with at this site to prevent lethal systemic infection. Although so far no leukocytes or cytokines have been identified in the worm, fine control of immunological homeostasis in the worm intestinal microenvironment is probably nevertheless essential for survival, as maintenance of local tissue integrity is required for efficient nutrient absorption. Some data indicate that mammalian innate immunity is regulated by the nervous system, but the mechanisms linking the nervous and innate immune systems have so far remained shrouded in mystery[6].

Neuronal and behavioral studies constitute intriguing and profound areas of *C. elegans* research[7]. Notably, this hermaphrodite worm has only 302 neurons, all of whose connections are known[7]. Many functional circuits have been identified, and many neurons and/or neuronally important genes can be eliminated while viability is still maintained[7]. Consequently, *C. elegans* provides an excellent model that should represent a prototype for understanding the complex interactions between the nervous and immune systems.

In this issue of *Nature Immunology*, Kawli and Tan provide convincing evidence for a neuronal control mechanism that suppresses innate immunity at the site of PA14 infection[3]. They demonstrate that loss of expression in neurons of either syntaxin or calcium-activator protein for secretion enhances the resistance of worms to PA14 infection, probably through inhibition of the release of

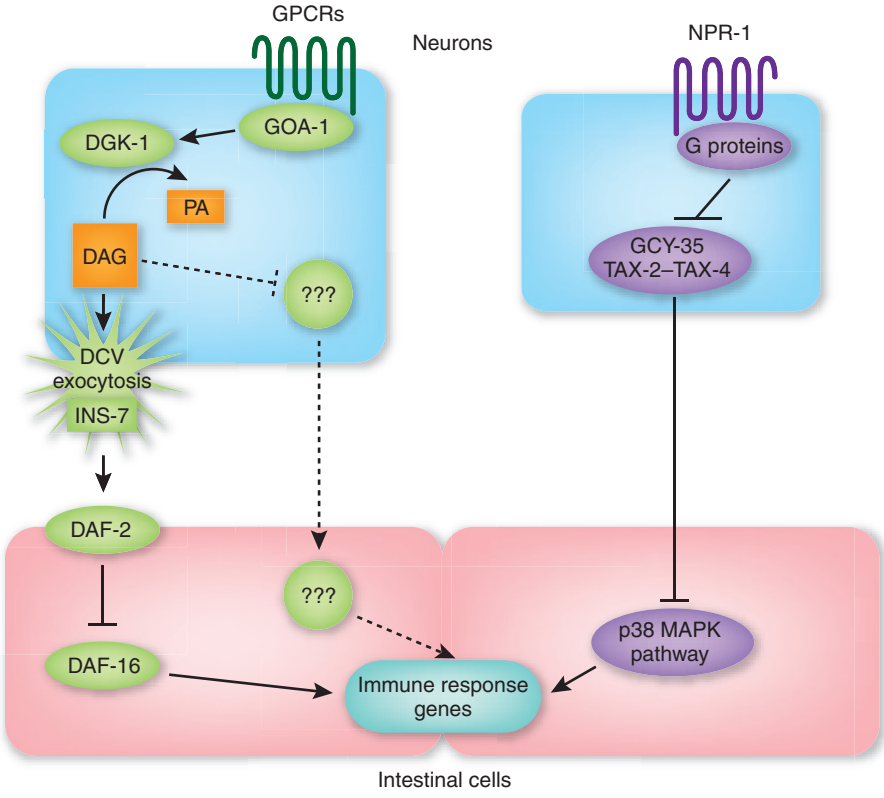

Figure 1 Model for neuronal regulation of the innate immune response of intestinal cells to bacterial infection. Left, insulin and neuropeptides secreted from neuronal DCVs activate DAF-2 and other factors in the intestine, which causes inhibition of DAF-16. Neuronal GOA-1 relays a signal that diminishes DCV exocytosis by decreasing diacylglycerol (DAG) concentrations through the kinase DGK-1; these signals consequently lead to activation of DAF-16, which promotes the expression of immunity-related genes and resistance to bacterial infection. In addition, an as-yet-unidentified DAF-2- and DAF-16-independent pathway 'downstream' of GOA-1 and diacylglycerol contributes to this regulation. Right, neuronal NPR-1 inhibits the GCY-35 and TAX-2–TAX-4 pathway, which in turn inhibits activation of the intestinal p38 MAPK pathway (simplified model). It is possible that NPR-1 is the GPCR 'upstream' of GOA-1. PA, phosphatidic acid.

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neuronal dense core vesicles (DCVs). They further show that loss of the GOA-1 $G_{o\alpha}$ subunit or diacylglycerol kinase, which results in more neurotransmission, renders worms hypersensitive to PA14. From their data they infer that release of DCVs from neurons normally acts to suppress innate immunity to PA14. In agreement with their conclusion, release of DCVs decreases the expression of immunity-related genes in the intestine. As the DAF-2–DAF-16 insulin-signaling pathway has been shown to be important in the worm immune response to PA14 infection[8], Kawli and Tan test whether this pathway is influenced by neuronal release of DCVs. The data show that the resistance associated with loss of neuronal release of DCVs is mediated by the DAF-2–DAF-16 insulin pathway operating in the intestine. Moreover, removal of the insulin-like peptide INS-7 results in the same phenotype as does inhibition of DCV secretion, and expression of INS-7 exclusively in neurons is sufficient to restore wild-type protection in an INS-7-mutant worm. Thus, INS-7 provides a potential link between DCVs released from neurons and the DAF-2–DAF-16–mediated innate immune response in the intestine. Thus, this work provides a plausible model of how secretion signals in neurons can regulate innate immune responses at sites of infection.

In *Science*, Styer *et al.* provide independent evidence of a link between the intestinal innate immunity of *C. elegans* against PA14 and the nervous system[4]. Using a similar model of PA14 infection of *C. elegans*, they show that loss of NPR-1, a G protein–coupled receptor (GPCR) similar to mammalian neuropeptide Y receptors, enhances susceptibility to PA14. A considerable portion, but not all, of this susceptibility could be accounted for by changes in how NPR-1-mutant worms behave on 'lawns' of PA14. They further show that NPR-1 acts through GCY-35, a soluble guanylate cyclase, and the TAX-2 and TAX-4 components of a cGMP-gated ion channel, as loss of GCY-35, TAX-2 or TAX-4 suppresses the PA14-hypersensitive phenotype of NPR-1–loss-of-function mutants. Styer *et al.* further show that NPR-1 functions in three sensory neurons (AQR, PQR and URX) for its effect on innate immunity and that NPR-1 is important for the activation of intestinal immunity-related genes 'downstream' of PMK-1. However, as it is not clear if these last experiments were done in conditions in which the 'behavioral' aspects of the immune response could be separated from 'nonbehavioral' aspects, further work is needed to determine the mechanism(s) by which NPR-1 influences the innate immune response to PA14.

These findings collectively indicate a common theme for non–cell autonomous control of innate immunity by the nervous system (**Fig. 1**). As with all new and exciting observations that challenge or expand present paradigms, such studies raise new and interesting questions. What is/are the GPCR(s) that act 'upstream' of the GOA-1 $G_{o\alpha}$ subunit highlighted by Kawli and Tan? How might NPR-1 signaling lead to the non–cell autonomous activation of innate immune responses indicated by Styer *et al.*? Could these pathways be interconnected? As NPR-1 is a GPCR and mutation in its gene triggers the same phenotype as would mutation in the gene of the GPCR 'upstream' of GOA-1, it plausible that NPR-1 may be a GPCR coupled to GOA-1 in the pathway described by Kawli and Tan.

An unexplained puzzle is why DCV secretion has evolved to dampen the immune system in the presence of a pathogen. It could be that the DCVs themselves suppress and/or balance specific immune response genes to prevent overstimulation of the immune system and that, in the wild, such a balance is important for reasons not clear in the laboratory. It is also possible that PA14 has evolved to take advantage of this situation. *P. aeruginosa* stimulates the production of INS-7, and this greater production might increase the presence of INS-7 in DCVs. In this way, *P. aeruginosa* would ensure that when DCVs are released, they contain a large amount of the DAF-2 agonist INS-7 that will suppress intestinal innate immunity and therefore promote infection. One prediction of this model is that not all pathogens should induce INS-7 expression and that INS-7 induction might be a specific adaptation of *P. aeruginosa*. Such an observation has been reported before[9].

If the past is an accurate predictor of the future, then this *C. elegans* work linking control of innate immunity to the nervous system will have profound consequences and will 'translate' into a better understanding of host immune responses in other, more complex organisms. It is anticipated that the full biological importance of this regulation is just beginning to emerge.

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