



Review Article

Neuro-immune communication in *C. elegans* defense against pathogen infection

Phillip Wibisono, Jingru Sun*

Department of Biomedical Sciences, Elson S. Floyd College of Medicine, Washington State University, Spokane, WA, USA



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ABSTRACT

The innate immune system is a complex collection of physical barriers and physiological defense responses to internal and external environmental assaults. Recent studies in the model organism *Caenorhabditis elegans* have highlighted how the nervous system interacts with the innate immune system to generate coordinated protective responses. Indeed, studies on neuro-immune interaction pathways have provided mechanistic insights into the roles of neuro-immune communication in modulating both immune and behavioral responses to pathogen attacks. The nervous system releases a variety of neurotransmitters, peptides, and hormones that regulate the innate immune response, while the innate immune system also relays information to the nervous system to affect learning and behavioral responses. Although these interactions still need further investigation, the knowledge that we have gained thus far has improved our understanding of how separate biological systems can act collectively for the survival and well-being of an organism. Here, we review recent studies on neuro-immune communication related to the survival and defense of *C. elegans* against pathogens.

1. Introduction

The constant threat of pathogen infection is a major force driving the evolution of the immune system to detect and eliminate invading microorganisms efficiently and reliably. In vertebrates, the immune system consists of two separate but complementary pieces: the innate and adaptive immune systems. The innate immune system is a complex organization of physical barriers (epithelia of the skin, gastrointestinal tract, and respiratory system), antimicrobial peptides, secreted communication proteins, and professional cells (neutrophils, mast cells, natural killer cells, monocytes, and macrophages). The adaptive immune system, on the other hand, is a flexible collection of specialized professional cells (helper T-cells, cytotoxic T-cells, memory T-cells, and B-cells) that are responsible for the generation of immune memory and are key for immunization. The innate immune system is viewed as the first line of defense that differentiates self from invaders primarily by recognizing conserved *pathogen associated molecular patterns* (PAMPs) (Turvey and Broide, 2010), while the adaptive immune system is regarded as the source of specific defense responses due to its ability to retain memories of previously encountered pathogens.

Interestingly, most invertebrates, which make up the majority of animals on Earth, lack adaptive immunity and rely solely on their innate

immune systems to defend themselves against pathogen infection. The immune systems of these organisms consist of highly conserved features, which points to a shared origin dating back over 500 million years ago (Pancer and Cooper, 2006). Such conserved molecular pathways that provide protection against pathogen infection include, but are not limited to, the mitogen activated protein kinase (MAPK) pathway, the insulin-like growth factor (IGF) pathway, the transforming growth factor beta (TGF- β) pathway, and the NF- κ B pathway (Garsin et al., 2003; Kim et al., 2002; Mallo et al., 2002; Valanne et al., 2011; Imler and Hoffmann, 2002; Pees et al., 2016; Boehnisch et al., 2011; Huffman et al., 2004; Li et al., 2014; Schulenburg et al., 2008). Studying these pathways in model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* have advanced our understanding of how the innate immune system defends against a variety of pathogens (Mallo et al., 2002; Alper et al., 2007; Treitz et al., 2015; Shiratsuchi et al., 2012; Sahu et al., 2012; Irazoqui et al., 2010a; Wong et al., 2007; Riera Romo et al., 2016; Ermolaeva and Schumacher, 2014).

In this review, we describe research in the *C. elegans* field that focuses on the host defense response in relation to the nervous system. Other reviews have already broadly covered the worm's immune response and the various immune effectors (Schulenburg et al., 2004, 2008; Irazoqui et al., 2010b; Shivers et al., 2008; Kim and Ewbank, 2018); thus, here, we

* Corresponding author.

E-mail address: jingru.sun@wsu.edu (J. Sun).<https://doi.org/10.1016/j.crimmu.2021.04.002>

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aim to emphasize how *C. elegans* produces regulated defense responses utilizing the nervous system.

2. *C. elegans*, a simple yet powerful model for studying neuro-immune communication

The connection between the nervous and innate immune systems has become more evident since their communication was found to be evolutionarily conserved (Tracey, 2014). Studies in mammalian models have already shown that the nervous system can regulate the immune system through specific neuropeptides and other signaling molecules (Tracey, 2002; Steinberg et al., 2016; Cardoso et al., 2017). However, an imposing obstacle to extensively studying this communication in vertebrates is the excessive size and scope of parsing out the interactions between neurons. The human brain alone consists of approximately 86 billion neurons, with an equal number of non-neuronal support cells (Azevedo et al., 2009). This vast sea of synapses and gap junctions makes studying neuro-immune interactions in humans difficult with current technology. Luckily, these interactions are not exclusive to vertebrate organisms, allowing for meaningful basic research in simpler and more defined models. *C. elegans* is a prime model for such research with a total of only 302 neurons in hermaphrodites and 385 neurons in males. In addition to the small neuron count, the synaptic connections between neurons in *C. elegans* have been fully explored and mapped. These factors make *C. elegans* an excellent model for examining the roles of specific neurons and neuronal circuits in immune regulation during pathogen infection (Riddle et al., 1997).

C. elegans has been used to study conserved immune response pathways since the first of such studies were performed in 1999 (Maha-jan-Miklos et al., 1999; Tan et al., 1999a, 1999b). The characteristics of the worm, such as its short lifespan, its ability to self-fertilize, and the ease of genetic manipulation make it an ideal organism for high-throughput investigations of host-pathogen interactions. However, this model is not without its limitations. While *C. elegans* possesses many conserved immune signaling pathways, it lacks the inducible nitric oxide production response as well as the conserved Toll-like receptor (TLR) pathways found in other invertebrates and vertebrates. Indeed, its sole TLR protein, TOL-1, is not involved in the activation of the innate immune response but rather plays roles in development and avoidance behavior (Irazoqui et al., 2010b; Brandt and Ringstad, 2015). Furthermore, the worm does not have functional homologs of the transcription factor NF- κ B or the TLR adaptor protein MYD88 to mount a defense against pathogens (Pujol et al., 2001; Kurz and Ewbank, 2003). This absence of conventional immune activators and sensory proteins means that *C. elegans* probably lacks the more traditional rapid immune response signaling found in other animals, but at the same time, this model allows for studies of rapid neuro-immune communication without the confounding variability. The highly organized and rapid neuro-immune communication is a prime target for research on how *C. elegans* is able to modulate its immune response and how this model system can provide insight into the complex world of vertebrate neuro-immune communication (Steinberg et al., 2016; Kawli et al., 2010; Cao and Aballay, 2016; Cao et al., 2017).

3. Neural regulation of immune responses

The most immediate connection that *C. elegans* has to the outside environment is its sensory neurons located in the head of the animal. Sensing the outside world, these neurons govern behavioral responses to nutrient fluctuations using multiple biosynthetic pathways, including the posttranslational addition of O-linked N acetylglucosamine (O-GlcNAc) to serine and threonine residues. Inappropriate levels of O-GlcNAcylation have been linked to metabolic disease, while proper cycling of O-GlcNAc at gene promoters can regulate the stress response in *C. elegans* (Bond and Hanover, 2013; Love et al., 2010). Examination of the survival of mutants for OGT-1 and OGA-1, which are enzymes expressed in both head

neurons and the intestine and are required for the removal and addition of O-GlcNAc (Guo et al., 2014; Oranth et al., 2018), found that these mutants were hypersusceptible to killing by *Staphylococcus aureus* but not *Pseudomonas aeruginosa*. Moreover, whole-transcriptome analysis of these mutants identified defense genes that were significantly down-regulated during *S. aureus* infection, but the response to *P. aeruginosa* was unaffected. In addition, the induction of the immune response to *S. aureus* was found to be independent of p38/PMK-1 MAPK activation. These results indicate that by utilizing the O-GlcNAc nutrient sensing system, *C. elegans* is able to distinguish nearby bacteria and activate pathogen-specific defense responses (Bond et al., 2014).

The G Protein-Coupled Receptor (GPCR) Neuropeptide Receptor 1 (NPR-1) is expressed in multiple neurons and promotes survival against several pathogens by inhibiting AQR, PQR, and URX neurons (Styer et al., 2008). Styer et al. reported that *C. elegans* lacking NPR-1 showed a significant decrease in survival against *P. aeruginosa*, a phenotype that was attributed to both altered pathogen avoidance and decreased innate immunity (Styer et al., 2008). Animals with mutant *npr-1* also had mis-regulated gene expression in the p38/PMK-1 MAPK pathway in response to *P. aeruginosa*. Ablation of AQR, PQR, or URX neurons partially rescued the increased susceptibility of *npr-1* mutant animals to *P. aeruginosa*, and re-expression of NPR-1 in these neurons also rescued the *npr-1* mutant phenotype. These findings establish that the nervous system regulates the innate immune response during pathogen infection and highlight the role of neuronal GPCRs in controlling the p38/PMK-1 MAPK pathway. Similar to what Styer et al. discovered, Reddy et al. (2009) found that both the wild isolate CB4856 with a polymorphism in the *npr-1* gene and animals with a loss-of-function mutation in *npr-1* had enhanced susceptibility to *P. aeruginosa* when compared to wild-type N2 animals. However, they concluded that this difference is only caused by the oxygen-dependent behavioral avoidance but not by the direct regulation of innate immunity based on the observation that N2, CB4856, and *npr-1* mutants exhibited similar survival against *P. aeruginosa* in full-lawn assays and at lower oxygen concentrations (Reddy et al., 2009). Despite these differences, both studies suggest that there is an NPR-1-dependent neural circuit regulating the survival of *C. elegans* against *P. aeruginosa*.

Another neuronal GPCR, Neuropeptide Receptor 8 (NPR-8), expressed in the amphid neurons AWB, AWC, and ASJ, was shown to suppress *C. elegans* survival against pathogen infection by inhibiting the expression of cuticle collagen (Sellegounder et al., 2019). Functional loss of NPR-8 conferred enhanced survival against *P. aeruginosa*, while not affecting avoidance behavior or bacterial sensation (Sellegounder et al., 2019). Interestingly, unlike NPR-1, NPR-8 does not play a role in regulating conserved innate immune pathways. Transgenic rescues of *npr-8* using a pan-neuronal promoter or cell-specific promoters for AWB, AWC, or ASJ neurons in *npr-8* null animals restored collagen expression to wild type levels. All rescues of *npr-8* in neurons also suppressed the enhanced resistant phenotype of *npr-8* mutants to *P. aeruginosa*. Electron microscopy further revealed that the cuticle of *npr-8* mutant animals was more resistant to thinning and wrinkling when compared to wild-type animals during infection. This is consistent with the overexpression of collagen genes in *npr-8* mutants, as collagen is a key component in maintaining the animal's cuticle. Taken together, NPR-8 in AWB, AWC, and ASJ neurons acts redundantly to suppress the expression of multiple collagen genes to maintain cuticular homeostasis. Inactivation of *npr-8* increases the overall expression of these collagen genes and leads to the reinforcement of the cuticle against pathogen infection (Sellegounder et al., 2019). This challenges the prevailing view that the cuticle is a static physical barrier by presenting the cuticle as a dynamic structure regulated by the nervous system during pathogen infection.

The catecholamine GPCR Octopamine Receptor 1 (OCTR-1), which is expressed in neurons including the amphid neurons ASH and ASI, has also been shown to suppress the immune response in *C. elegans* (Sun et al., 2011, 2012; Sellegounder et al., 2018; Liu et al., 2016). Indeed, an *octr-1* null mutation conferred enhanced resistance to *P. aeruginosa* without affecting the animal's avoidance behavior (Sun et al., 2011). Animals

lacking ASH neurons also exhibited enhanced resistance to *P. aeruginosa*, similar to the *oct-1* mutants, while ASI neuron-deficient animals displayed defective avoidance behavior (Cao et al., 2017). Moreover, ablation of the octopamine-producing RIC interneurons showed similar enhanced resistance to *P. aeruginosa* due to the lack of conversion of tyramine to octopamine by tyramine- β -hydroxylase (Sellegounder et al., 2018). These results show that ASH and RIC neurons must act in concert to suppress the immune response during infection, adding another layer of control. Microarray experiments further revealed a significant number of genes upregulated in *oct-1* mutant animals. These included genes in the insulin-like/DAF-2, p38/PMK-1 MAPK, and CED-1 pathways. Inactivating either *pmk-1* or *ced-1* abolished the enhanced resistance phenotype of *oct-1* mutant animals, but a lack of *daf-16* did not affect the survival of the mutants. The CED-1 pathway also includes the Activated in Blocked UPR (*abu*) class of genes that are predominantly expressed in pharyngeal and intestinal tissues (Sun et al., 2011; Haskins et al., 2008). Half of the upregulated genes in the *oct-1* mutants that were associated with CED-1 were from this family, which indicates that OCTR-1 helps manage endoplasmic reticulum stress by suppressing the *abu* family of genes in the innate immune response to *P. aeruginosa* (Sun et al., 2011, 2012). This cascade of interactions during infection shows the complex order of events that occur between neurons and non-neuronal tissues to mediate immune and protein responses in a timely manner.

The neuronal regulator *olrn-1* expressed in the AWC neurons promotes homeostasis by suppressing the activation of the p38/PMK-1 MAPK pathway. When *olrn-1*, which normally controls the olfactory receptors in AWC during development, was mutated to a non-functional state, the animals exhibited enhanced resistance to *P. aeruginosa* (Foster et al., 2020). This enhanced survival was also correlated to an increased expression of immune response genes in the intestine. Extrachromosomal rescue of *olrn-1* in AWC fully suppressed the increased survival in null mutant animals, suggesting that *olrn-1* function in AWC to regulate the innate immune response (Foster et al., 2020). *olrn-1*'s connection to the olfactory receptor development and the immune response bridges neuro-immune regulation and nematode development.

Dopamine released by CEP neurons also suppresses *C. elegans* survival against *P. aeruginosa* by inhibiting the activation of the p38/PMK-1 MAPK immune pathway (Cao and Aballay, 2016). Animals with a loss-of-function mutation in the dopamine receptor *dop-4* displayed enhanced resistance to *P. aeruginosa* (Cao and Aballay, 2016). Animals lacking CEP neurons exhibited a similar phenotype to the *dop-4* mutants, and ablation of CEP neurons in *dop-4* mutants did not alter the mutants' survival against *P. aeruginosa*, indicating that dopaminergic CEP neurons play a key role in releasing dopamine to modulate the immune response. ASG neurons, which express DOP-4, were also required for the suppression of PMK-1, and single neuron rescue of DOP-4 in ASG neurons partially suppressed the enhanced resistance to *P. aeruginosa*. While dopamine is viewed as a therapeutic target for nervous system disorders, this research points to dopamine as a potential target for treating infectious diseases and immune disorders.

Similar to dopamine, serotonin inhibits the immune response of *C. elegans* to *Microbacterium nematophilum* (Anderson et al., 2013). The deformed anal region (DAR) phenotype is a visual indicator of the immune response in epithelial cells during *M. nematophilum* infection. Animals treated with exogenous serotonin did not display this phenotype, whereas animals with a null mutation in tryptophan hydroxylase (*tph-1*), an enzyme critical for serotonin biosynthesis, displayed an increased proportion of DAR phenotypes. Since TPH-1 is expressed in neurons, cell-specific rescues of *tph-1* in ADF or NSM neurons found that only ADF rescue restored the DAR phenotype to wild-type levels during pathogen infection. Further work showed that serotonin released by ADF neurons interacts with the G protein GOA-1 to inhibit the immune response by blocking EGL-30 in the anal region and, in turn, reducing survival. Taken together, the chemosensory ADF neurons in the head of the animal are able to suppress the immune response in the distal tail region, dependent upon environmental cues (Anderson et al., 2013).

Cholinergic signaling also regulates the innate immune response in the intestine against *S. aureus* infection (Laped et al., 2018). Using an RNA interference screen of 890 GPCR genes, the authors found that silencing *gar-2* and *gar-3*, genes that encode for muscarinic Acetylcholine (ACh) receptors, suppressed the survival of *C. elegans* against *S. aureus*. Activation of these receptors using an ACh-mimic induced the expression of immune defense genes. This induction could be suppressed by silencing *gar-2* and *gar-3*, indicating that the expression of the immune defense genes in the intestine requires ACh activation (Laped et al., 2018). The authors further showed that the release of ACh from the nervous system to the intestine activates the muscarinic receptors, which, in turn, triggers the transcription factor LIN-1 to express Wnt and its receptor Frizzles. This cascade of signaling leads to the induction of immune response genes and overall increase in survival against *S. aureus*.

The nervous system in this context provides prompt communication pathways for *C. elegans* to modulate immune responses. Such regulation by the nervous system allows for the rapid expression of activator ligands to mount a defense response or suppressors to maintain protein homeostasis in the event of pathogen challenge.

4. Aversive behavior response

Avoidance and physical distancing are simple, yet effective defense strategies employed by *C. elegans* to improve survival against pathogen attacks (Chang et al., 2011; Turner et al., 2020; Meisel and Kim, 2014; Martin et al., 2017). *C. elegans* has both innate aversion to pathogens and the ability to learn avoidance behavior with prior exposure (Zhang et al., 2005; Tran et al., 2017). Chemosensory neurons and interneurons play key roles in both of these processes. The amphid chemosensory neurons AWB and AWC have been implicated in sensing food odors and play roles in the attraction and repulsion behaviors of *C. elegans*. Using intracellular calcium imaging, both neuron pairs were observed to respond to the odors of *Escherichia coli* OP50 and *P. aeruginosa* PA14 (Ha et al., 2010; Chalasani et al., 2007). Initially, naive *C. elegans* prefer the scent of *P. aeruginosa*, as opposed to the standard food source *E. coli* OP50; however, after a short exposure time, the animals begin to avoid *P. aeruginosa* (Ha et al., 2010). The neural circuit required for recognizing and showing preference between the two bacteria was further elucidated using laser ablation of the neurons downstream of AWB and AWC (Ha et al., 2010). It is only after nitric oxide produced by *P. aeruginosa* activates ASJ neurons do the animals begin to avoid the bacterial lawn. Accordingly, ablation of ASJ neurons or a null mutation of *tax-4*, a sensory transduction channel subunit gene, abolished the avoidance behavior against *P. aeruginosa*. Further molecular investigations found that DAF-11 and Guanylyl Cyclase 27 (GCY-27) mediated the detection of nitric oxide by ASJ neurons. S-nitrosylation of cysteine residues in both DAF-11 and GCY-27 was shown to activate the GC catalytic domain of these proteins and further signal denitrosylating enzymes, such as the thioredoxin TRX-1. The induction of *trx-1* in ASJ neurons changes the activation response of the neurons to a biphasic response that triggers an avoidance response behavior (Hao et al., 2018). Therefore, *C. elegans*, unlike most other terrestrial animals, can sense nitric oxide produced by bacteria, which, in turn, can provoke a behavior change in the nematode. However, this learned response can also be induced in other ways since intestinal bloating during bacterial infection can promote pathogen avoidance to *P. aeruginosa* PA14 (Singh and Aballay, 2019a). Distension of the gut by bacterial colonization induces a response in the neuroendocrine pathway in an NPR-1- and DAF-7-dependent manner. Indeed, loss of the NPR-1 ligands FLP-18 and FLP-21 resulted in complete suppression of the enhanced avoidance behavior, while DAF-7 mutants showed significant but partial suppression (Singh and Aballay, 2019a).

As stated above, the sole Toll-like Receptor in *C. elegans*, TOL-1, is not directly linked to activation of the immune response, in contrast to what is seen in other animals. However, TOL-1 was shown to be required for the avoidance response to *Serratia marcescens* (Pujol et al., 2001). TOL-1 is expressed in the chemosensory BAG neurons and interacts with the

signaling proteins More Of Ms 4 (MOM-4) and PMK-3 to suppress I κ B and promote CO₂ avoidance. Accordingly, disruption of TOL-1 or PMK-3 was shown to affect the animal's ability to sense *S. marcescens* (Brandt and Ringstad, 2015). BAG neurons sense hypoxia by a distinct mechanism requiring the heteromeric guanylated cyclases GCY-31 and GCY-33 (Zimmer et al., 2009). However, *gcy-31* mutant animals retained their robust avoidance behavior to *S. marcescens*. Heat-killed or attenuated strains of *S. marcescens* failed to produce an avoidance response in *C. elegans*. Animals lacking GCY-9, which are CO₂ blind (Hallem et al., 2011), also failed to respond to *S. marcescens*. Further studies showed that a mixture of CO₂ and odorant produced by *S. marcescens* induced strong avoidance behavior and the activation of BAG neurons. Taken together, these results suggest that *C. elegans* can use CO₂ sensing to maximize feeding options while minimizing exposure to active pathogens (Brandt and Ringstad, 2015).

ADF neurons, a pair of chemosensory neurons, promote aversive behavior by increasing the levels of serotonin after pathogen exposure. Correspondingly, animals with ablated or non-functioning ADF neurons lack the avoidance response (Shivers et al., 2009). Interestingly, serotonin in ADF neurons has been linked to learning avoidance behavior, which is achieved through the serotonin-gated chloride channel MOD-1 to reinforce negative behavior in the animals (Zhang et al., 2005). Similarly, AVA neurons, a pair of interneurons, affect aversive learning through the TGF- β pathway (Zhang and Zhang, 2012). AVA neurons produce the ligand DBL-1, which binds its receptor SMA-6 in both ASI neurons and the hypodermis to promote aversive learning in response to *P. aeruginosa* (Zhang and Zhang, 2012).

ASJ neurons, another pair of chemosensory neurons, were found to be part of the DAF-7/TGF- β signaling pathway for avoidance towards *P. aeruginosa* (Meisel et al., 2014). Phenazine-1-carboxamide and pyochelin, two secondary metabolites produced by *P. aeruginosa*, are able to activate G protein signaling in ASJ which, in turn, activates the TGF- β pathway by expression of DAF-7. The TGF- β pathway stimulates the nearby neurons RIM/RIC and promotes an avoidance response behavior against *P. aeruginosa* (Meisel et al., 2014). In contrast to these results, Singh and Aballay (2019b) reported that the presence of phenazine was not sufficient for the induction of an avoidance response. Moreover, they showed that the avoidance response was induced not by chemosensation but rather bloating of the intestine and that this behavior was regulated by both DAF-7/TGF- β and NPR-1 signaling (Singh and Aballay, 2019b). They also showed that these pathways controlled aerotaxis and lead to aversive learning toward *P. aeruginosa*'s lower oxygen concentration versus *E. coli*'s relatively higher oxygen levels (Singh and Aballay, 2019b). While these studies present conflicting mechanisms, they both illustrate that the avoidance behavior is mediated by the nervous system through a system of environmental and molecular cues.

ASI neurons promote learning by releasing insulin-like peptides (Chen et al., 2013). Animals with mutations in *daf-28* that disrupt the cleavage and folding of proteins are defective in learning (Chen et al., 2013). Two ligands similar to DAF-28, INS-6 and INS-7, were examined for their influence on learned pathogen avoidance. INS-6 expressed in ASI neurons was found to repress the expression of *ins-7* in URX neurons. INS-7 acts on the RIA neurons, binding antagonistically to DAF-2 to inhibit the learning circuit. With INS-7 being repressed by the binding of INS-6 to URX neurons, DAF-2 activity is uninhibited in the RIA neurons, which promotes aversive learning (Chen et al., 2013).

More recent research has shown that the aversive learning towards *P. aeruginosa* can not only be retained for the lifetime of trained animals but can also be passed down transgenerationally. Indeed, *C. elegans* exposed to *P. aeruginosa* learns to avoid the bacteria, and then this avoidance behavior, which is specific to *P. aeruginosa*, can be observed in the following generations, without previous exposure, for up to four generations (Moore et al., 2019). This aversive learning utilizes the TGF- β ligand DAF-7 in ASI neurons because RNAi silencing of *daf-7* was shown to abolish the inherited behavior in subsequent generations (Moore et al.,

2019). Exposure to *P. aeruginosa* changes the levels of a large group of non-coding small Piwi-interacting RNAs (piRNAs) that are necessary for the inheritance of the transgenerational pathogen avoidance behavior. These piRNAs lead to the creation of secondary siRNAs that direct chromatin modifications via histone modifying enzymes. The modifications lead to the continued upregulation of DAF-7 in ASI neurons, which, in turn, promotes the avoidance behavior in response to *P. aeruginosa* exposure (Shivers et al., 2009). This transgenerational learning mediated by the TGF- β and piRNA pathways provides the progeny of pathogen-challenged animals with a survival advantage while navigating a complex environment.

Interestingly, whole bacteria are not required for the induction of avoidance to *P. aeruginosa* PA14. Animals exposed to bacterial non-coding small RNAs (sRNAs) of less than 200 nt showed avoidance to live *P. aeruginosa*, and treating such RNA with RNase abolished this behavior (Kaletsky et al., 2020). Exposure to *P. aeruginosa* PA14 sRNAs also resulted in upregulated DAF-7 expression in both ASI and ASJ neurons, and a functioning RNAi pathway was shown to be required to induce the subsequent avoidance behavior. Similar to the learned aversion to live bacteria, this sRNA-derived behavior is also inherited transgenerationally (Kaletsky et al., 2020).

In a reversal of roles, the expression of immune-related genes was shown to influence behavior in *C. elegans* during injury and infection. Following injury or infection, the innate immune response is activated through a MAPK/TGF- β -dependent cascade, causing the expression of Neuropeptide-Like Proteins (NLPs) and CaeNaCins (CNCs) (Sinner et al., 2021). In particular, NLP-29 binds to its receptor NPR-12 in RIM and PVC neurons, which triggers the downstream RIS neurons to stimulate sleep in the animals. Impairing this response to injury by inactivating the *aptf-1* gene or multiple *nlp/cnc* genes caused the risk of death post-injury to triple. This suggests that the induction of sleep by the expression of immune genes is key for survival, and that neuro-immune communication can work in both directions for the survival of an organism (Sinner et al., 2021).

5. Outlook

Use of the *C. elegans* model has greatly improved our understanding of how the nervous and immune systems interact with each other to enable the host to survive an ever-shifting pathogenic environment. The nervous system regulates aversive behavior to avoid pathogen attacks as well as the innate immune response to fight infection when an attack does occur. Because *C. elegans* lacks some of the evolutionarily conserved immune sensory proteins required to initiate defense responses in large vertebrates, future studies may find that the *C. elegans* nervous system plays an indispensable role in pathogen sensing. In addition to regulating the activation of immune signaling pathways upon pathogen infection, the nervous system likely also regulates the resolution of the immune response upon pathogen clearance, as the nervous and immune systems are so intertwined in function. Studies in *C. elegans* revealed that the nervous system also controls the basal expression of immune genes under noninfectious conditions to maintain immune homeostasis (Cao and Aballay, 2016; Sellegounder et al., 2018; Foster et al., 2020; Kawli and Tan, 2008). These neuro-immune interactions may ultimately influence aging and longevity, as these biological processes share many molecules, cells and signaling pathways (Kim, 2013). Overall, using *C. elegans* as a simple model to investigate neuro-immune communication pathways may help researchers parse out possible confounding variables in studies of immune or neurological disorders in humans and other vertebrates.

CRedit authorship contribution statement

Phillip Wibisono: Conceptualization, Writing – original draft, and, revision. **Jingru Sun:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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