Immune defense mechanisms in the *Caenorhabditis elegans* intestinal epithelium

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Abstract

Intestinal epithelial cells provide an essential line of defense for *Caenorhabditis elegans* against ingested pathogens. Because nematodes consume microorganisms as their food source, there has presumably been selection pressure to evolve and maintain immune defense mechanisms within the intestinal epithelium. Here we review recent advances that further define the immune signaling network within these cells and suggest mechanisms used by the nematode to monitor for infection. In reviewing studies of pathogenesis that use this simple model system, we hope to illustrate some of the basic principles of epithelial immunity that may also be of relevance in higher order hosts.

Introduction

The coordinated regulation of immune defenses is of paramount importance to combat infection and prevent the deleterious effects of unchecked immune responses on host cells. This is of particular importance in the mammalian intestine where potential pathogens must be differentiated from innocuous bacterial species that are part of the normal flora of humans. One approach to understand the mechanisms of immune detection at epithelial surfaces is to use invertebrate hosts, such as the microscopic nematode *Caenorhabditis elegans*, to examine evolutionarily conserved aspects of innate immunity and pathogen virulence [1–13]. In its natural habitats, *C. elegans* animals consume bacteria and fungi as their food source and encounter numerous threats from ingested microorganisms. Thus, there has presumably been a strong selection pressure to evolve and maintain a defense system within the intestinal epithelium of the nematode that is able to mount targeted defense responses toward pathogens, but not against innocuous food sources.

Because of the wealth of genetic and genomic tools available for *C. elegans* research, we are beginning to understand immune mechanisms in the nematode, which has offered important insights into the origins and fundamental principles of immunity. This topic has been reviewed previously on a number of occasions [1–13], most recently in 2010 [7]. In this review, we focus on studies that use *C. elegans* to study the immune mechanisms in intestinal epithelial cells and highlight several exciting developments that have emerged in the last two years.

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Key differences between the nematode and vertebrate innate immune systems

Several key features of the mammalian innate immune response are not encoded in the *C. elegans* genome, including homologs of the transcription factor NF-κB or the Toll-like receptor (TLR) adaptor protein MYD88. In addition, the sole TLR homolog in *C. elegans* does not appear to play a major role in activating the innate immune response by functioning either directly or indirectly as a receptor for pathogen-associated molecular pattern (PAMP) molecules. *C. elegans* also does not produce homologs of the known vertebrate cytokines. Interestingly, many of these prominent features of the mammalian immune response appear to have been lost from the nematode lineage during evolution since they are present in more primitive metazoans such as the sea anemone *Nematostella vectensis*, suggesting that the common eumetazoan ancestor of cnidarian (sea anemone and hydra) and bilaterian (worms, arthropods, vertebrates) metazoans had these innate immune signaling components [7]. What then can we learn from the study of *C. elegans* innate immunity? Although the nematode lacks NF-κB, MYD88 and other components of the TLR signaling pathway, it mounts an immune response that utilizes several evolutionarily conserved signaling pathways, including p38 mitogen-activated protein kinase (MAPK), β-catenin, and FOXO transcription factors, which surprisingly appear to function in parallel to activate at least partially non-overlapping sets of effector genes. A question of primary importance to immunologists is whether these conserved *C. elegans* immune signaling pathways are also involved in the mammalian innate immune response. From this perspective, it can be argued that nematodes offer an excellent opportunity to identify TLR-independent and NF-κB-independent features of the metazoan innate immune response that may be difficult to identify and study in vertebrate models.

C. elegans anatomy facilitates study of host–pathogen interactions in the intestine

C. elegans does not have an adaptive immune system or mobile immune cells, such as professional phagocytes. Thus, defenses mounted by intestinal epithelial cells are critically important to defend the nematode against ingested pathogens. Importantly, key anatomical features of the *C. elegans* intestinal epithelium are conserved in mammals (Figure 1). Both cell types have a polarized structure with apical microvilli attached to a terminal web composed of actin and intermediate filaments. Moreover, the nematode is transparent, which allows direct microscopic observation of invading pathogens in an intact host. Finally, the worm intestine in its entirety consists of only 20 non-renewable cells, greatly simplifying the analysis of an entire infectious process, which can be monitored in real time using a variety of microscopic techniques. Two groups have recently taken advantage of these features of *C. elegans* intestinal cell anatomy to study novel, naturally occurring pathogens of nematodes.

Troemel et al. identified a microsporidial pathogen in a wild-caught *C. elegans* strain isolated from a compost pit near Paris, France, which they found comprised a new genus and species [14••,15,16]. This organism, named *Nematocida parisii*, establishes an intracellular infection within the intestinal epithelium of the nematode and eventually kills the animal. Infected nematodes actively shed spores into the environment, which cause infection in neighboring animals. By direct visualization of infected nematodes in which the actin cytoskeleton and terminal web were engineered to express YFP or CFP, respectively, Estes *et al.* show that *N. parisii* creates gaps in the normally contiguous terminal web by promoting the cellular redistribution of actin toward the basolateral side of the cell away from its normal apical location in the terminal web and microvilli [14••]. They postulate that *N. parisii* spores exit through these gaps into the intestinal lumen.

In another important study of pathogens identified in wild-caught nematodes, Félix *et al.* characterized the first two viruses able to infect *Caenorhabditis* species [17••]. From animals with unusual morphological features in their intestines, these researchers identified two single-stranded RNA viruses that are distantly related to nodaviruses and capable of infecting a variety of laboratory nematode strains. Interestingly, host RNAi machinery was implicated in the defense against these viruses. The mechanisms used by *C. elegans* epithelial cells to defend against intracellular pathogens are incompletely understood, but are now the subject of focused investigations using these new and interesting natural *C. elegans* pathogens.

The *C. elegans* p38 MAP kinase PMK-1: one central regulator, multiple immune outputs

Principal among the immune regulators in *C. elegans* is the NSY-1/SEK-1/PMK-1 MAP kinase pathway, which was identified in a forward genetic screen for mutants with enhanced susceptibility to infection with the Gram-negative bacteria Pseudomonas aeruginosa [18]. This pathway is orthologous to the ASK1 (MAP kinase kinase kinase)/MKK3/6 (MAP kinase kinase)/p38 (MAP kinase) pathway in mammals, and its identification in *C. elegans* provided an important clue about the evolutionary origins of innate immunity. Activation of this signaling cassette is complex. An ortholog of mammalian SARM called Toll-interleukin-1 receptor (TIR-1) [19–22], and the protein kinases Cδ (PKCδ) [23] and D (PKD) [24] act upstream of NSY-1. A recent study found that a signaling module formed by the G protein alpha subunit (Gqα) and the signal transducer phospholipase Cβ (PLCβ) modulate the activity of the p38 MAP kinase cassette within the intestine [25••]. Interestingly, however, stimulation of the p38 MAP kinase cassette occurs in a manner independent of the single TLR homolog in *C. elegans* (*tol-1*). Thus, dissection of the p38 MAP kinase cassette enables analyses of immune mechanisms that are important in the absence of TLR signaling.

The p38 MAP kinase pathway acts cell autonomously in the intestinal epithelium [26] to coordinate defense against a wide variety of ingested pathogens. *C. elegans* carrying loss-of-function mutations in *pmk-1* are hypersusceptible to infection with the Gram-negative pathogens *P. aeruginosa* [18,27], *Salmonella enterica* [28], *Yersinia pestis* [29•] and *Serratia marcescens* [30••]; the Gram-positive pathogens *Enterococcus faecalis* [30••] and *Staphylococcus aureus* [31]; and the fungus *Candida albicans* [32••]. Moreover, activity of the p38 MAP kinase PMK-1 declines with age and was recently shown to underlie the increased susceptibility to bacterial killing that occurs in older *C. elegans* [33••]. Troemel *et al.* used global transcriptional profiling analyses of nematodes growing under normal laboratory conditions to show that PMK-1 regulates the expression of putative antimicrobial effectors, including ShK toxins, C-type lectins and genes carrying a CUB-like domain [27], in the absence of pathogen challenge. This has been termed 'basal regulation,' to distinguish it from the induction of immune effectors that occurs during challenge with a pathogen. In addition, a variety of genes that are induced during pathogen attack by diverse pathogens require PMK-1 [18,27,29•,32••]; however, the full spectrum of genes that are activated by pathogens in a PMK-1-dependent manner has not been determined. Interestingly, transcriptional profiling experiments have demonstrated that the immune effectors upregulated by divergent pathogens, including several genes that require PMK-1, are largely non-overlapping [27,29•,32••,34••]. These data suggest that the PMK-1 cascade coordinates the induction of multiple immune effectors that differ depending on the infecting organism.

Work by Shivers *et al.* has recently shed light on a mechanism downstream of PMK-1 that accounts for part of the immune specificity mediated by this protein [30••]. Using an approach that highlights some of the advantages of working with a model genetic host such

as *C. elegans*, these researchers fused the promoter for a PMK-1-dependent putative antimicrobial peptide with the gene encoding GFP and integrated the array into the *C. elegans* genome, thereby creating an *in vivo* sensor for the transcriptional activation of this gene. By conducting a forward genetic screen for *C. elegans* mutants that were both hypersusceptible to *P. aeruginosa* infection and exhibited diminished expression of this transcriptional reporter, they uncovered mutations in each of the four genes in the p38 MAP kinase cassette (TIR-1, NSY-1, SEK-1 and PMK-1) and also in ATF-7, a transcription factor orthologous to mammalian ATF2/ATF7, which did not previously have a described immune function in *C. elegans*. Subsequent characterization of ATF-7 in the *C. elegans* antibacterial immune response revealed that it functions as a repressor of p38 MAP kinase PMK-1-dependent genes in *C. elegans* when worms are feeding on *E. coli*. However, ATF-7 switches to become a transcriptional activator of immune response genes when it is directly phosphorylated by PMK-1 during *P. aeruginosa* infection. Interestingly, it seems that ATF-7 is not a positive regulator of resistance to *E. faecalis*, despite the fact that PMK-1 is required for normal defense against this Gram-positive pathogen. These data suggest that there are PMK-1-dependent signaling regulators that are downstream of PMK-1 and independent of ATF-7 that are differentially activated during *E. faecalis* infection. How one pathway coordinates such disparate outputs remains an open and very interesting question.

Evolutionarily ancient mechanisms of pathogen detection

A remarkable feature of *C. elegans* innate immunity that has emerged from a number of transcriptional profiling experiments is that the nematode is able to mount pathogen-specific immune responses. The genes induced by infection with *P. aeruginosa* [27], *S. aureus* [34••], *Microbacterium nematophilum* [35], and *C. albicans* [32••] are remarkably distinct. Moreover, we recently found that the nematode selectively represses the transcription of putative antibacterial immune effectors during infection with the pathogenic fungus *C. albicans* [32••], an observation that was supported by a separate study of two nematode fungal pathogens [36•]. Marsh *et al.* also reported that a single antimicrobial peptide required for normal defense against a fungal pathogen of the nematode acts as a susceptibility factor for a bacterial pathogen [37•]. Taken together, these data imply the existence of mechanisms that enable the nematode to distinguish between invading microbes to coordinate pathogen-specific defense responses.

Transcriptional profiling data have revealed that *C. elegans* induces the transcription of putative defense effectors following exposure to heat-killed, avirulent *C. albicans* and *S. aureus* [32••,34••]. These data suggest that surveillance for invading microorganisms in the nematode may be mediated, at least in part, through 'pattern recognition,' an ancient immune surveillance mechanism able to detect conserved microbial molecules [so-called microbial-associated or pathogen-associated molecular patterns (MAMPs/PAMPs)] [38]. However, no direct evidence that *C. elegans* can detect MAMPs/PAMPs has been published. Interestingly, pattern recognition may not play as important a role in the detection of *P. aeruginosa* infection. Heat-killed *P. aeruginosa* fail to elicit an immune response [34••] and the induction of a particular anti-pseudomonal defense gene was dependent on the pathogenic potential of the infecting bacterial strain [39••]. In addition, unpublished experiments from the Troemel and Ausubel laboratories suggest that *C. elegans* may monitor the host effects of bacterial toxins to trigger an immune response. However, the detailed mechanisms by which *C. elegans* monitors and responds to *P. aeruginosa* infection are still not known. They may involve context-dependent signals generated during the infection, which in other systems have been called 'DAMPs' [40–42] or 'patterns-of-pathogenesis' [43]. In mammals, molecules released from damaged tissues including DNA, uric acid, and ATP have been shown to activate innate immunity [40]. Although some details are beginning to emerge [44–46], the receptors and downstream regulators involved

in the detection and response to DAMPs have not yet been identified. Interestingly, plants have evolved somewhat analogous mechanisms of immune defense that rely heavily on indirect recognition of pathogen invasion. In addition to surveying for MAMPs/PAMPs, plants utilize a family of conserved intracellular receptor structurally related to NOD-like receptors (NLRs) in mammals [47] to monitor the activities of pathogen-encoded molecules, so-called 'effectors,' and activate an immune response when host-derived target molecules are modified [48]. Evidence for such 'effector-triggered immunity' is also beginning to emerge in other metazoans, but not yet in *C. elegans* [49]. The genetic, genomic, and morphological features of *C. elegans* summarized above make the nematode a powerful model in which to further dissect conserved mechanisms of metazoan pathogen detection.

The unfolded protein response during bacterial infection

Several investigators have demonstrated a specific role for endoplasmic reticulum (ER) unfolded protein response (UPR) pathways in the intestine during infection of the nematode [50••,51••,52,53••]. Richardson *et al.* found that p38 MAP kinase PMK-1-mediated defenses require compensatory activation of the UPR by the X-box binding protein (XBP-1) to handle the accumulation of unfolded proteins in the ER, which occurs as the host mounts an immune response that is comprised primarily of secreted genes [51••]. In a separate study, these investigators also demonstrated a dynamic requirement for the UPR in the maintenance of cellular homeostasis [54]. The UPR in *C. elegans* is also required for normal defense against pore-forming toxins produced by pathogenic bacteria [52]. Interestingly, p38 MAP kinase PMK-1 and c-Jun N-Terminal Kinase (JNK)-like MAP kinase form a signaling network that regulates both the UPR and other cellular defenses following exposure to pore-forming toxins [53••]. In addition, Sun *et al.* found that inputs from the sensory nervous system in the nematode suppress innate immune responses, which they conclude occurs via the downregulation of the UPR in non-neuronal tissues [50••]. It also seems that these sensory neurons receive signals from pathogenic, but not heat-killed bacteria, which raises the intriguing hypothesis that they detect some pattern of pathogenesis to fine-tune the host immune response during infection. An interesting body of literature suggests that the nervous system also regulates a pathogen avoidance program that confers a survival advantage for nematodes during infection [55–57]. The precise roles that neuronal signaling pathways play in the regulation of immune signaling pathways, on the one hand, and pathogen avoidance on the other, have not been fully resolved [57–59]. A detailed discussion of the neural control of immunity and behavioral avoidance of pathogens is outside the scope of this review.

Parallel pathways and the evolution of innate immune defenses

The p38 MAP kinase pathway coordinates the basal and infection-induced regulation of immune effectors that are required for defense against most *C. elegans* pathogens, but it does not act alone (Figure 2). The transcription factor ZIP-2 controls an immune signaling pathway that acts independently of PMK-1 and is induced by only by virulent strains of *P. aeruginosa* [39••]. *C. elegans* FSHR-1 is a G-protein coupled receptor and homolog of the mammalian follicle-stimulating hormone receptor controls an immune signaling pathway in *C. elegans* that is also distinct from the PMK-1 pathway [60]. Likewise, the β-catenin homolog BAR-1 and the homeobox transcription factor EGL-5 are important for defense against *S. aureus* [34••,61]. A third pathway regulated by the FOXO transcription factor DAF-16 acts downstream of the insulin/insulin-like growth factor receptor DAF-2 to regulate longevity, immunity, and stress resistance [27,62–64]. A recent report presents data that the conserved transcription factor SKN-1, an ortholog of mammalian Nrf proteins, coordinates a transcriptional program that protects the host from its own reactive oxygen species, which are produced to fight bacterial infection of the nematode [65,66]. Using

slightly different assay conditions, Shivers *et al.*, however, did not find that SKN-1 was required for defense against *P. aeruginosa* [30••]. Each of these pathways acts in parallel to coordinate the elaboration of largely non-overlapping immune effectors. We hypothesize that defense pathways in *C. elegans* evolved in response to environmental threats, further dissection of which may yield clues about evolutionarily conserved immune mechanisms in higher order hosts.

Novel anti-infective compounds identified using a *C. elegans* pathogenesis assay

One potentially interesting application of *C. elegans* pathogenesis assays involves their use in large-scale screens to identify novel antimicrobials [12]. Such assays are facilitated by the fact that 15–20 adult *C. elegans* animals fit comfortably in the wells of standard 384-well assay plates and can be lethally infected with a variety of microbial pathogens [1–13]. In the first relatively high throughput study of this kind, Moy *et al.* tested 37,214 compounds in a high-throughput assay and identified 119 small molecules that prolonged the lifespan of nematodes infected with the Gram-positive human bacterial pathogen *E. faecalis*, including a number that had no structural relationship to any known antimicrobials [67]. Interestingly, several of these small molecules cured nematodes at doses lower than required to inhibit bacterial growth. In contrast, traditional antibiotics, such as ciprofloxacin, ampicillin and vancomycin cured *E. faecalis*-infected nematodes only at doses several fold higher than the *in vitro* minimum inhibitory concentration (MIC) for the bacteria. These data raise the intriguing possibility that a subset of the small molecules identified in the *C. elegans*-based screen act either by inhibiting virulence factor production in the bacteria or by directly stimulating the host innate immune response. Work is currently underway to characterize the mechanism of action of these interesting compounds.

Conclusions

Dissection of the mechanisms of host defense and pathogen detection in *C. elegans* holds promise to elucidate the origins and fundamental principles of innate immunity, and may lead to important developments that broaden our understanding of such processes in higher order hosts.

Acknowledgments

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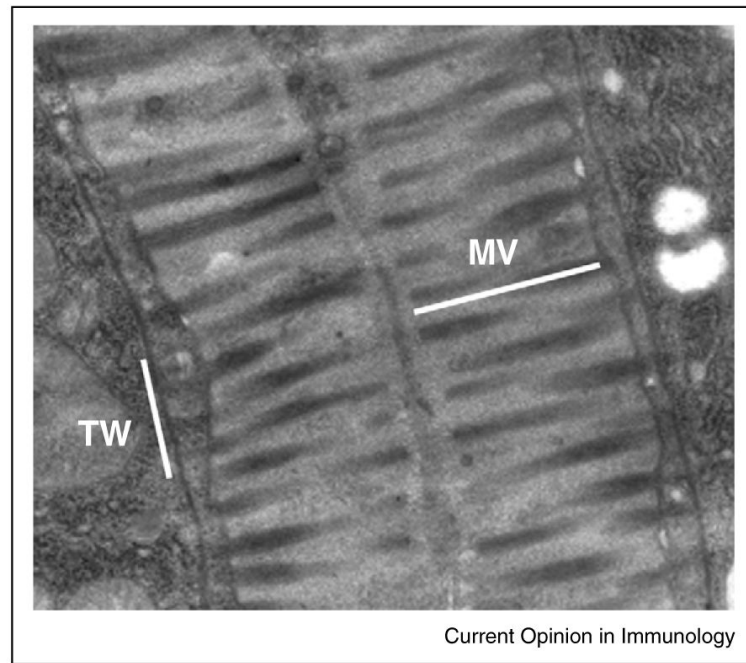
Figure 1.
Features of *C. elegans* intestinal epithelial cells are strongly conserved. *C. elegans* intestinal epithelial cells bear a striking resemblance to human intestinal cells. Both cells have a brush border composed of microvilli (MV) anchored to a cellular structure called the terminal web (TW).This image was originally published by Troemel *et al.* [15] and is reproduced here with permission from Emily Troemel.
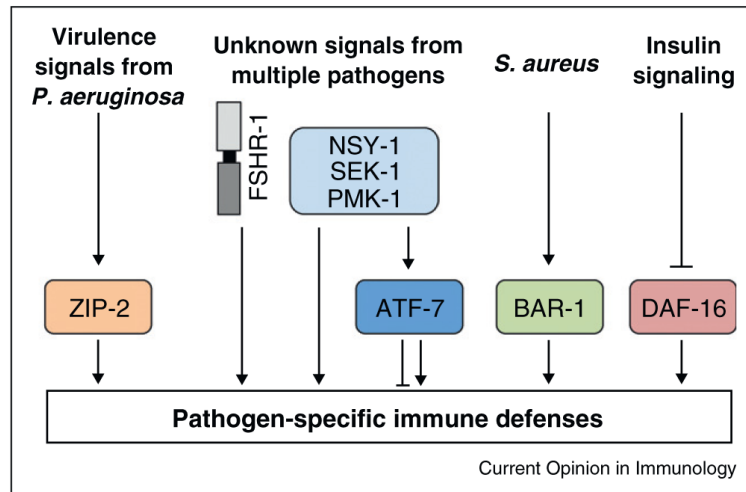
Figure 2.
Multiple immune signaling pathways function in *C. elegans* epithelium. *C. elegans* coordinate pathogen-specific immune responses toward ingested pathogens through several signaling pathways that act in parallel. The transcriptional outputs from these signaling mediators are unique, but can be overlapping.