Innate Immunity in Caenorhabditis elegans Is Regulated by Neurons Expressing NPR-1/GPCR

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A large body of evidence indicates that metazoan innate immunity is regulated by the nervous system, but the mechanisms involved in the process and the biological importance of such control remain unclear. We show that a neural circuit involving npr-1, which encodes a G protein–coupled receptor (GPCR) related to mammalian neuropeptide Y receptors, functions to suppress innate immune responses. The immune inhibitory function requires a guanine 3′,5′-monophosphate-gated ion channel encoded by tax-2 and tax-4 as well as the soluble guanylate cyclase gcy-35. Furthermore, we show that npr-1– and gcy-35–expressing sensory neurons actively suppress immune responses of nonneuronal tissues. A full-genome microarray analysis on animals with altered neural function due to mutation in npr-1 shows an enrichment in genes that are markers of innate immune response genes, including those regulated by a conserved PMK-1/p38 mitogen-activated protein kinase signaling pathway. These results present evidence that neurons directly control innate immunity in C. elegans, suggesting that GPCRs may participate in neural circuits that receive inputs from either pathogens or infected sites and integrate them to coordinate appropriate immune responses.

Innate immune defense comprises a variety of mechanisms used by metazoans to prevent microbial infections. Activation of the innate immune system upon pathogen recognition results in a rapid and definitive microbicidal response to invading microorganisms that is fine-tuned to prevent deleterious deficiencies or excesses in the response. The nervous system, which can respond in milliseconds to many types of non-specific environmental stimuli, has several characteristics that make it an ideal partner with the innate immune system to regulate nonspecific host defenses (1–3). However, even though a large body of evidence indicates that metazoan innate immunity is under the control of the nervous system, the mechanisms involved in the process and the biological importance of such control remain unclear. To provide insights into the neural mechanisms that regulate innate immunity, we have taken advantage of the simple and well-studied nervous and innate immune systems of Caenorhabditis elegans.

The powerful genetic approaches available to C. elegans research have been used to address central questions concerning the functions of the nervous system (4). With its 302 neurons and 56 glial cells, which represent 37% of all somatic cells in a hermaphrodite, the nervous system is perhaps the most complex organ of C. elegans. Ablation of different neurons has demonstrated that sensory neurons regulate a variety of physiological processes, including dauer formation and adult life span (5–8). In addition, C. elegans neurons are known to express numerous secreted peptides of the transforming growth factor-β (TGF-β) family, the insulin family, and neuropeptide families (6, 9–13). This myriad of secreted factors has the potential to act at a distance to modulate various physiological processes by regulating the function of neuronal and nonneuronal cells throughout the animal.

Like other free-living nematodes, C. elegans lives in soil environments where it is in contact with soilborne microbes, including human microbial pathogens; it has evolved physiological mechanisms to respond to different pathogens by activating the expression of innate immune response genes that are conserved across metazoans (14–19). C. elegans also has behavioral responses to pathogenic bacteria such as Bacillus thuringiensis (20, 21), Microbacterium nematophilum (22), Photorhabdus luminescens (23), Pseudomonas aeruginosa (24–26), and Serratia marcescens (24, 27, 28). Animals infected with these pathogens avoid lawns of the pathogen, or migrate away from pathogen odors. It is currently unknown how the nematode can sense pathogenic bacteria, although mutants in sensory-transduction molecules such as the Gi-like protein ODR-3 and the G protein–coupled receptor kinase GRK-2 are incapable of S. marcescens lawn avoidance (28). These results suggest that G protein–coupled receptors (GPCRs) may participate in neural circuits that receive inputs from either pathogens or infected sites and integrate them to coordinate appropriate defense responses.

To study the role of GPCRs in the regulation of innate immune response, we first determined the susceptibility of 40 C. elegans strains carrying mutations in GPCRs to the human opportunistic pathogen P. aeruginosa strain PA14, a clinical isolate capable of rapidly killing C. elegans at 25°C
C. elegans GPCR NPR-1 is involved in immunity to P. aeruginosa. (A) C. elegans strains carrying mutations in GPCRs were screened for altered survival on P. aeruginosa. C56G3.1(ok1439) (P = 0.0347), F57H12.4(ok1504) (P = 0.0071), and str-182(ok1419) (P = 0.0342) had enhanced resistance to P. aeruginosa, and npr-1(ad609) (P = 0.0246) had enhanced susceptibility to P. aeruginosa, relative to the wild type (N2). Shown is the time required for 50% of the nematodes to die (TD50) as mean ± SEM corresponding to at least three independent experiments, each of which used at least 40 adult nematodes per strain. (B) Wild-type N2 and npr-1(ad609) (P = 0.0001) nematodes were exposed to P. aeruginosa and scored for survival over time. The graph represents combined results of four independent experiments (n ≥ 40 adult nematodes per strain). (C) Wild-type N2 and npr-1(ad609) (P = 0.1411) nematodes were exposed to heat-killed P. aeruginosa and scored for survival over time. The graph represents the combined results of two independent experiments (n = 100 adult nematodes per strain). (D) Wild-type N2, npr-1(ad609) (P = 0.0001), npr-1(ky13) (P = 0.0001), npr-1(n1353) (P = 0.0001), npr-1(ur89) (P = 0.0001), npr-1(g320) (P = 0.0001), and the wild isolate npr-1(g320)-WI (P = 0.0922) were exposed to P. aeruginosa and scored for survival over time. Shown is a representative assay of at least three independent experiments (n = 48 adult nematodes per strain).

Fig. 2. Hyperoxia avoidance of NPR-1-deficient animals increases susceptibility to P. aeruginosa. (A) C. elegans wild-type N2 animals and (B) npr-1(ad609) mutants were propagated at 20°C as hermaphrodites on modified nematode growth agar plates seeded with E. coli strain OP50 and then visualized using a MZ FLIII stereomicroscope (Leica, Bannockburn, Illinois). The characteristic aggregate of npr-1(ad609) nematodes shown here is at the edge of the bacterial lawn. (C) Wild-type N2 and (D) npr-1(ad609) nematodes (n = 12 each) were exposed to P. aeruginosa for 24 hours under standard killing assay conditions and visualized using a MZ FLIII stereomicroscope. Under these conditions, npr-1(ad609) nematodes do not form characteristic aggregates of the strain. (E) Wild-type N2 and npr-1(ad609) nematodes were exposed to either a full lawn or a center lawn of P. aeruginosa on a 3.5-cm-diameter plate and scored for survival over time. Under both conditions, npr-1(ad609) animals were more susceptible to P. aeruginosa-mediated killing (P = 0.0001). Wild-type animals on full lawns were more susceptible to P. aeruginosa-mediated killing than animals on center lawns (P = 0.0001); npr-1(ad609) animals were equally susceptible (P = 0.07). The graph represents combined results of three independent experiments (n ≥ 40 adult nematodes per strain). (F) Wild-type N2 and npr-1(ad609) nematodes exposed to P. aeruginosa in either 21% or 8% oxygen at room temperature (20°C to 23°C) and scored for survival over time. Under both conditions, npr-1(ad609) animals were more susceptible to P. aeruginosa-mediated killing (P = 0.0001). npr-1(ad609) animals in 21% oxygen were more susceptible to P. aeruginosa-mediated killing than were animals in 8% oxygen (P = 0.0001); wild-type animals were equally susceptible (P = 0.95). The graph represents combined results of two independent experiments (n = 40 adult nematodes per strain).
wild type, the npr-1(g320) allele conveys susceptibility to P. aeruginosa in an N2 background (Fig. 1D). These results suggest that the German isolate may have evolved a mechanism to compensate for the increased susceptibility to pathogens because of its reduced NPR-1 activity.

To determine whether the immune deficiency due to mutation in the npr-1 gene is specific for P. aeruginosa infection, we exposed npr-1(ad609) nematodes to Salmonella enterica and Enterococcus faecalis, two human pathogens known to kill C. elegans (32–34). As shown in Fig. S3, A and B, npr-1(ad609) nematodes exhibited an enhanced susceptibility to these pathogens, suggesting that NPR-1 is required for immune responses to pathogens in general.

NPR-1 is involved in a neural circuit that integrates behavioral responses to environmental oxygen, food, and other animals. In nature, NPR-1 is found in two allelic forms that differ in a single amino acid at position 215, NPR-1(215F) and NPR-1(215SF) (35). The NPR1(215SF) allele, which is found in the standard laboratory strain, has high activity, whereas the NPR1(215F) allele has low activity (35, 36). Wild-type npr-1(215F) animals avoid oxygen levels above 10% when food is absent, but fail to avoid high oxygen in the presence of Escherichia coli bacteria, the food provided to C. elegans in the laboratory. In contrast, npr-1(215F) and npr-1 animals carrying loss-of-function (lf) alleles have strong hypoxia avoidance in the absence or presence of E. coli (37). As a result, npr-1(215F) and npr-1(215SF) show a preference for the thickest part of a bacterial lawn, the region in which oxygen levels are the lowest (35). In addition, because nematode aggregation into feeding groups decreases local oxygen concentrations, npr-1(215F) and npr-1(215SF) form aggregates of nematodes when the animals are grown at densities high enough to allow this behavioral response (37).

One potential explanation for the reduced life span of npr-1(215F) mutants grown on bacterial pathogens is that aggregation increases nematode susceptibility to pathogen infection. However, the animal density in the assays where the susceptibility to pathogens is tested was not sufficient to elicit aggregation, making this possibility unlikely (Fig. 2D). Even though npr-1(ad609) animals did not aggregate, they still exhibited a preference for the thickest part of the lawn, where oxygen concentrations are lower (Fig. 2, C and D). In addition, long-term exposure to P. aeruginosa caused wild-type animals to leave the bacterial lawn, a potentially protective behavioral response, but leaving was not observed in npr-1(ad609) animals. Thus, we examined whether the behavior of npr-1(ad609) animals could affect susceptibility to pathogens. The number of bacterial cells in npr-1(ad609) animals was not found to be greater than that in wild-type animals (Fig. S2) at early stages of the infection, suggesting that the increased susceptibility to pathogens of npr-1(ad609) animals is not caused by a higher dose of bacteria. In addition, we grew animals on agar plates that were completely covered in P. aeruginosa, a condition that eliminates both the lawn border (favored by npr-1 animals) and the ability to leave the lawn (favored by wild-type animals). As shown in Fig. 2E, wild-type animals grown on plates completely covered by P. aeruginosa died at a higher rate than did animals grown on plates containing a small lawn of P. aeruginosa in the center of the plate. npr-1(ad609) animals were equally susceptible to P. aeruginosa when grown on full or center lawns. Together, these results indicate that the lawn-avoiding behavior of wild-type animals contributes to their increased survival. However, npr-1(ad609) animals still exhibited enhanced susceptibility to P. aeruginosa relative to the wild type when the infections were performed in plates containing full lawns (Fig. 2E). These results indicate that lawn avoidance is part of the C. elegans defense response to P. aeruginosa but cannot account for all of the differences between wild-type and npr-1(ad609) animals.

To determine whether other elements of the oxygen response contribute to the enhanced susceptibility of npr-1(ad609) nematodes, we compared animals grown in 21% oxygen with those grown in 8% oxygen, a favorable oxygen environment that suppresses most behavioral phenotypes of npr-1 mutants. Under 8% oxygen, npr-1(ad609) animals do not exhibit a preference for the bacterial border and are capable of leaving the P. aeruginosa lawn. As shown in Fig. 2F, npr-1(ad609) animals were more resistant to
that passes through the pharyngeal grinder proliferates and colonizes the intestine in a process that is independent of the dose (32), and the pumping rates of npr-1(ad609) animals are comparable with those of the wild type (fig. S4), the results further support the function of NPR-1 in the regulation of immune responses.

Genetic studies have identified a chemosensory circuit that coordinates oxygen preference and aggregation in npr-1 mutants (35, 37, 39–42). Aggregation and bordering of npr-1(ad609) nematodes depend on functional gcy-35, tax-2, or tax-4 genes (31, 40, 43). GCY-35 is a soluble guanylyl cyclase (sGC) that binds directly to molecular oxygen, and TAX-2 and TAX-4 are two subunits of a guanosine 3',5'-monophosphate–gated ion channel (31, 34, 43). Through the activity of GCY-35 and other guanylate cyclases and the subsequent activation of TAX-2/TAX-4, the AQR, PQR, and URX sensory neurons drive avoidance of high oxygen; these neurons are thought to be hyperactive in npr-1 mutants (40). To determine whether this part of the NPR-1 neural circuit regulates innate immune response, we studied the pathogen susceptibility of npr-1(ad609) animals carrying loss-of-function mutations in gcy-35,
NPR-1 is expressed in at least 20 different neurons, including the gcy-35–expressing sensory neurons AQR, PQR, and URX (35). To confirm that at least AQR, PQR, and URX neurons are part of a neural network that inhibits innate immunity, we studied the susceptibility to P. aeruginosa of a strain in which these neurons were genetically ablated by expressing the cell-death activator geneegl-1 under the control of the gcy-36 promoter (42). The strain lacking AQR, PQR, and URX neurons exhibited a significantly increased survival on P. aeruginosa (Fig. 3D), indicating that AQR, PQR, and URX neurons suppress innate immunity. In addition, lack of AQR, PQR, and URX neurons partially rescued the enhanced susceptibility to P. aeruginosa of npr-1(ad609) animals (Fig. 3D). Expression of npr-1 under the control of the gcy-32 promoter, which drives the expression of npr-1 to the AQR, PQR, and URX neurons, also rescued the enhanced susceptibility to P. aeruginosa of npr-1(ad609) animals (Fig. 3E), providing additional evidence of the role of these neurons in the regulation of innate immunity. Consistent with the idea that additional NPR-1–expressing neurons regulate innate immunity (35), npr-1 expression under the regulation of its own promoter fully rescued the altered expression of 10 out of 19 genes tested that are markers of the C. elegans immune response. These results indicate that the NPR-1 neural circuit modulates the expression of immune-related genes, many of which are known to be expressed in tissues that are in direct contact with pathogens during infection. Our results provide evidence that specific genes and neurons in the nervous system are responsible for effective innate immune responses that are independent of behavioral phenotypes and may take place in tissues that are in direct contact with pathogens. It has recently been postulated that cell–nonautonomous signals from different neurons may act on nonneural tissues to regulate processes such as fat storage (48) and longevity (8). C. elegans neurons can regulate physiological processes through conserved neuroendocrine signals, including insulin-related peptides, TGF-β peptides, and neuropeptides. The URX, AQR, and PQR neurons that are part of the NPR-1 neural circuit that regulates innate immunity are exposed to the pseudocelomic body fluid, which could communicate neuroendocrine signals to nonneural tissues involved in defense responses. The identification and characterization of the specific neuroendocrine signals that regulate innate immune responses in C. elegans should yield several insights into the mechanisms used by the nervous system to regulate similar processes across metazoans.

References and Notes
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