Endoplasmic Reticulum Stress Pathway Required for Immune Homeostasis Is Neuurally Controlled by Arrestin-1

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Background: GPCRs function in the C. elegans nervous system to control immunity.

Results: Arrestin-1, the only GPCR adaptor in C. elegans, functions in the nervous system to control immunity.

Conclusion: Neural arrestin-1 signal regulates immunity.

Significance: Our data underscore the importance of the nervous system in the control of longevity and immune homeostasis and suggest that overlapping and distinct neural circuits control these processes.

In response to pathogen infection, the host innate immune system activates microbial killing pathways and cellular stress pathways that need to be balanced because insufficient or excessive immune responses have deleterious consequences. Recent studies demonstrate that two G protein-coupled receptors (GPCRs) in the nervous system of Caenorhabditis elegans control immune homeostasis. To investigate further how GPCR signaling controls immune homeostasis at the organismal level, we studied arrestin-1 (ARR-1), which is the only GPCR adaptor protein in C. elegans. The results indicate that ARR-1 is required for GPCR signaling in ASH, ASI, AQR, PQR, and URX neurons, which control the unfolded protein response and a p38 mitogen-activated protein kinase signaling pathway required for innate immunity. ARR-1 activity also controlled immunity through ADF chemosensory and AFD thermosensory neurons that regulate longevity. Furthermore, we found that although ARR-1 played a key role in the control of immunity by AFD thermosensory neurons, it did not control longevity through these cells. However, ARR-1 partially controlled longevity through ADF neurons.

Increasing evidence indicates that activation of the innate immune system accounts for the major physiological, metabolic, and pathological responses to infections (1). The response to microbial infection is accompanied by increased demand for protein folding in the endoplasmic reticulum (ER), which must be alleviated by UPR pathways (2–8). The nervous system, which can respond in milliseconds to different environmental stimuli, has several characteristics that make it an ideal partner with the innate immune system to regulate nonspecific host defenses (9–11).

To provide insights into the neural mechanisms that regulate innate immunity, we have taken advantage of the simple immune system and the very well characterized nervous system of Caenorhabditis elegans. A unique advantage to the study of neural mechanisms involved in the control of immune responses in C. elegans is the unparalleled characterization of its nervous system at the cellular level. Each of the 302 neurons of an adult animal has a precise identity, and, most uniquely, C. elegans is the only animal for which the wiring diagram of the nervous system has been established based on anatomical reconstructions. NPR-1, a GPCR similar to mammalian neuropeptide Y receptors, participates in a neural circuit that controls the p38/PMK-1 MAPK pathway required for innate immunity (12). In addition, OCTR-1, which is a catecholamine GPCR for octopamine, controls not only the p38/PMK-1 MAPK pathway but also a canonical and a noncanonical UPR pathway that may play a key role to alleviate ER stress during immune responses against microbial pathogens (7, 8).

The discovery that individual GPCRs function in the nervous system of C. elegans to control the immune system (7, 8, 12) offers a unique opportunity to analyze the role of the entire nervous system in immune homeostasis. To study the role of neural GPCR signaling in the regulation of UPR genes and its role in immune defense against bacterial infections, we took advantage of a unique component of GPCR signaling, the GPCR adaptor protein arrestin-1 (ARR-1). Arrestins block G protein-mediated signaling and can also function as signal transducers in their own right (13). The only member of the arrestin family in C. elegans, ARR-1, is expressed almost exclusively within the C. elegans nervous system (14), making it easy to manipulate neural ARR-1 signaling and to study its effect on innate immune responses against pathogen infection.

Here we show that ARR-1 functions in a number of sensory neurons that regulate UPR genes that are expressed in nonneuronal tissues and required for immune defense. ARR-1 also partially controls longevity through ADF neurons. The results highlight the importance of ARR-1 signaling in a subset of sensory neurons that control longevity and immune homeostasis during response to pathogen infection.

EXPERIMENTAL PROCEDURES

Bacterial Strains—The following bacterial strains were used: Escherichia coli OP50 (15), Pseudomonas aeruginosa PA14 (16), Salmonella enterica serovar Typhimurium 1344 (17), Yersinia...
Neural Arrestin-1 Regulates Innate Immunity

**Nematode Strains**—The following *C. elegans* strains were cultured under standard conditions and fed *E. coli* OP50 (15). The Bristol N2 strain was used as wild type. N2, *arr-1 (ok401)*, *daf-16(mu86)*, *npr-1(ad609)*, and *octr-1(ok371)* strains were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis) and outcrossed at least three times. *arr-1 (ok401)*;*daf-16(mu86)*, *arr-1 (ok401);npr-1 (ad609)*, and *octr-1(ok371);arr-1 (ok401)* were constructed using standard genetic techniques.

**Plasmid Constructs and Generation of Transgenic Lines**—A 3.2-kb genomic fragment containing the *arr-1* gene was cloned into the BamHI and Smal sites of pPD95.77 vector (Fire Lab C. elegans Vector kit; Addgene, Cambridge, MA) creating a translational fusion between ARR-1 and GFP. Plasmid pVS3 was constructed by inserting 1924 bp of the *arr-1* promoter upstream of *arr-1::gfp* in pPD97.77. Plasmid pVS4 was constructed by inserting 4685 bp of the *unc-119* promoter upstream of *arr-1::gfp* to confer pan-neuronal expression (19). Plasmid pVSS was constructed by inserting 3045 bp of the *sra-6* promoter upstream of *arr-1::gfp* to confer expression in ASH and ASI neurons (20). Plasmid pVS6 was constructed by inserting 1095 bp of the *gcy-32* promoter upstream of *arr-1::gfp* to confer expression in AQR, PQR, and URX neurons (21).

Plasmid pVS7 was constructed by inserting 1472 bp of the *srl-142* promoter upstream of *arr-1::gfp* to confer expression in ADF neurons (20). Plasmid pVS8 was constructed by inserting 940 bp of the *gcy-8* promoter upstream of *arr-1::gfp* to confer expression in AFD neurons (22). *arr-1 (ok401)* animals were microinjected with the plasmids to generate transgenic strains AY103 *arr-1 (ok401)/pVS3(parr-1::arr-1::gfp);pRF4(rol-6(su1006))*; AY104 *arr-1 (ok401)/pVS4(punc-119::arr-1::gfp);pRF4(rol-6(su1006))*; AY105 *arr-1 (ok401)/pVSS(pscn-6::arr-1::gfp);pRF4(rol-6(su1006))*; AY106 *arr-1 (ok401)/pVS6(pgcy-32::arr-1::gfp);pRF4(rol-6(su1006))*; AY107 *arr-1 (ok401)/pVST(pshh-142::arr-1::gfp);pRF4(rol-6(su1006))*; and AY108 *arr-1 (ok401)/pVS8(pgcy-8::arr-1::gfp);pRF4(rol-6(su1006))* The plasmids were maintained as extrachromosomal arrays. The expression pattern of neuron-specific promoters was confirmed for all of the transgenic strains.

**C. elegans Killing Assay**—*C. elegans* wild-type N2 animals and mutants were maintained as hermaphrodites at 20 °C, grown on modified nematode growth medium (NGM) agar plates (0.35% instead of 0.25% peptone), and fed with *E. coli* OP50 as described. The bacterial lawns used for *C. elegans* killing assays were prepared by placing a 15-μl drop of an overnight culture of the bacterial strains on modified NGM agar on plates 3.5 cm in diameter. Full lawn plates used for *C. elegans* killing assays were prepared by spreading a 25-μl drop of an overnight culture grown at 37 °C of *P. aeruginosa* on the complete surface of modified NGM agar in 3.5-cm-diameter Petri plates. Plates were incubated at 37 °C for 12–16 h. Plates were cooled down at room temperature for at least 1 h before seeding with synchronized young adult animals. The killing assays were performed at 25 °C, and live animals were transferred daily to fresh plates. Animals were scored at the times indicated and were considered dead when they failed to respond to touch.

**C. elegans Lifespan Assay**—*E. coli* OP50 was grown as described above. A 50-μl drop of the bacteria was plated on a 6-cm plate of modified NGM agar containing 40 μg/ml fluoro-deoxyuridine from Sigma. The assays were performed at 20 °C.

**Profile of Bacterial Accumulation in the Nematode Intestine**—To determine the profiles of bacterial accumulation in the intestine, wild-type, *arr-1 (ok401)*, and *octr-1(ok371)* animals were synchronized. Synchronized L1 larvae were grown on *E. coli* OP50 at 20 °C until they had reached the young adult stage. Wild-type, *arr-1 (ok401)*, and *octr-1(ok371)* animals were then transferred to plates seeded with *P. aeruginosa* expressing GFP (*P. aeruginosa*/GFP) cultured for 24–40 h at 25 °C. Animals were transferred to an NGM plate seeded with *E. coli* for 15 min and transferred again to a new NGM plate seeded with *E. coli* for 30 min to eliminate *P. aeruginosa*/GFP stuck to the body of the nematodes. Animals were visualized and imaged using a Leica MZ FLIII fluorescence stereomicroscope.

**Quantification of Intestinal Bacterial Loads**—For the quantification of cfu, wild-type, *arr-1 (ok401)*, AY103, AY104, AY105, AY106, AY107, and AY108 animals were synchronized for 22 h in S basal liquid medium at room temperature. Synchronized L1 larval animals were placed onto NGM plates seeded with *E. coli* OP50 at 20 °C until they had reached the young adult stage. Wild-type, *arr-1 (ok401)*, and transgenic animals were then transferred to plates seeded with *P. aeruginosa*/GFP for 24 h at 25 °C. Animals were transferred to an NGM plate seeded with *E. coli* for 15 min to eliminate *P. aeruginosa*/GFP stuck to the body of the worms. Animals were transferred to a new NGM plate seeded with *E. coli* for 30 min to further eliminate external *P. aeruginosa*/GFP. Ten nematodes/condition were transferred into 50 μl of PBS plus 0.1% Triton X-100 and ground. Serial dilutions of the lysates (10⁻¹, 10⁻², 10⁻³, 10⁻⁴) were plated onto LB/kanamycin to select for *P. aeruginosa*/GFP cells and grown overnight at 37 °C. Single colonies were counted next day and represented as the number of bacterial cells or cfu per worm.

**Confocal Microscopy**—AY103 and AY104 worm strains were imaged using the 63× lens of a Leica TCS SL confocal microscope. To construct the whole worm, the individual images were layered and flattened in Photoshop CS5.

**RNA Isolation**—Gravid adult wild-type, *arr-1 (ok401)*, and the transgenic animals were lysed using a solution of sodium hydroxide and bleach (ratio of 5:2) and washed, and the eggs were synchronized for 22 h in S basal liquid medium at room temperature. Synchronized L1 larval animals were placed onto NGM plates seeded with *E. coli* OP50 and grown at 20 °C until the animals had reached the L4 larval stage. Animals were collected and washed with M9 buffer before transferring to NGM plates containing *P. aeruginosa* PA14 for 4 h at 25 °C. After 4 h, animals were collected and washed with M9 buffer, and RNA was extracted using TRIzol reagent (Invitrogen). Residual genomic DNA was removed by DNase treatment (Ambion, Austin, TX).

**Quantitative Real-time PCR (qRT-PCR)**—Total RNA was obtained as described above. qRT-PCR was conducted using the Applied Biosystems One-Step Real-time PCR protocol using gene-specific Taqman assays on an Applied Biosystems
ARR-1 Signaling Regulates Pathogen Resistance and Lifespan Extension by Targeting Different Pathways—We studied whether ARR-1 is required for C. elegans defense against bacterial infections by exposing arr-1(ok401) mutant animals to P. aeruginosa and by comparing their survival with that of wild-type animals. arr-1(ok401) animals exhibited an enhanced resistance to P. aeruginosa (ERP) phenotype (Fig. 1A), suggesting that the lack of inhibition of GPCR signaling in the entire nervous system due to a lack of ARR-1 activity enhances immunity. Wild-type and arr-1(ok401) animals exhibited similar pumping rates on P. aeruginosa, indicating that they are exposed to a comparable dose of pathogen (data not shown). To determine whether the enhanced immune response caused by mutation in the arr-1 gene is specific to P. aeruginosa, we exposed arr-1(ok401) animals to S. enterica and Y. pestis, two Gram-negative pathogens known to kill C. elegans (24, 25). As shown in Fig. 1, C and D, arr-1(ok401) animals also exhibited an enhanced resistance to these pathogens.

Because pathogen avoidance is part of the C. elegans defense response to P. aeruginosa that is controlled by the nervous system (12), we examined the ERP phenotype of arr-1(ok401) animals on agar plates that were completely covered in bacteria, a condition that eliminates pathogen avoidance. arr-1(ok401) animals died at a slower rate than wild-type animals (Fig. 1B), indicating that pathogen avoidance does not play a role in the ERP phenotype of arr-1(ok401) animals. Consistent with this conclusion, arr-1(ok401) animals are resistant to S. enterica (Fig. 1C), a pathogen that does not elicit an avoidance behavior (26).

The extended lifespan of arr-1(ok401) animals grown on bacterial pathogens may simply be a consequence of their extended lifespan when grown on nonpathogenic E. coli (27). However, arr-1(ok401) mutants were more susceptible to the Gram-positive bacterium Enterococcus faecalis than wild-type animals (data not shown), making this possibility unlikely. In addition, although inhibition of the FOXO family transcription factor DAF-16 that controls longevity fully suppressed the extended lifespan of arr-1(ok401) animals (Fig. 1E), it only slightly suppressed the ERP phenotype of arr-1(ok401) animals (Fig. 1F). These results suggest that ARR-1 regulates different pathways involved in pathogen resistance and lifespan extension.

Neural ARR-1 Regulates Innate Immunity against Bacterial Pathogens—Expression of ARR-1::GFP under its own promoter restored ARR-1 expression in the nervous system (Fig. 2C) and rescued the ERP phenotype of arr-1(ok401) animals (Fig. 2A), providing evidence for the role of neuronal ARR-1 signaling in the regulation of innate immunity. ARR-1::GFP expressed under the regulation of a pan-neuronal promoter unc-119 (Fig. 2F) also fully rescued the ERP phenotype of arr-1(ok401) animals (Fig. 2D). Native or pan-neuronal expression of ARR-1 also rescued the reduced accumulation of P. aeruginosa phenotype exhibited by arr-1(ok401) animals (Fig. 2B and E).

Role of ARR-1 Function in Neurons Expressing GPCRs That Control Innate Immunity—OCTR-1, a putative octopamine GPCR, functions in sensory neurons designated ASH and ASI to actively suppress immune defense against P. aeruginosa responses by down-regulating the expression of abu genes (7). These genes belong to a family of ppp (prion-like glutamine[Q]/ asparagine[N]-rich domain-bearing protein) genes that have been named abu (activated in blocked unfolded protein

![Figure 1](https://example.com/fig1.png)

**FIGURE 1.** ARR-1 regulates both pathogen resistance and lifespan extension. A–D, wild-type (WT) and arr-1(ok401) animals were exposed to P. aeruginosa (A), full lawn of P. aeruginosa (B), S. enterica (C), and Y. pestis (D) and scored for survival. E, WT versus arr-1(ok401) (p < 0.0001); F, WT versus arr-1(ok401); daf-16(mu86), and daf-16(mu86);arr-1(ok401) mutant animals were scored for lifespan. F, wild-type, arr-1(ok401), daf-16(mu86), and daf-16(mu86);arr-1(ok401) mutant animals were exposed to P. aeruginosa and scored for survival. A–D, differences were statistically significant in all cases (p values < 0.0001). E, WT versus arr-1(ok401) (p < 0.0001). F, arr-1(ok401) versus daf-16(mu86);arr-1(ok401) (p = 0.0205) are shown. Results represent assays of three to five independent experiments; n = 100–120 animals/strain.
Neural Arrestin-1 Regulates Innate Immunity

To study the role of ARR-1 in ASH and ASI neurons to regulate immune homeostasis, we studied arr-1(ok401) animals carrying a deletion in octr-1. The ERP phenotype of arr-1(ok401);octr-1(ok371) animals was stronger than that of arr-1(ok401) or octr-1(ok371) animals (Fig. 3A). In addition, unlike octr-1(ok371) animals, arr-1(ok401) animals are more resistant to bacterial accumulation (Fig. 3C and supplemental Fig. S2), indicating that the ERP phenotype of arr-1(ok401) animals is mediated through GPCRs other than OCTR-1. If OCTR-1 were the only GPCR blocked by ARR-1 in ASH and ASI neurons, the rescue of ARR-1 expression in these neurons would further enhance the ERP phenotype of arr-1(ok401) animals and the up-regulation of abu genes. However, no differences in survival or bacterial accumulation were observed between arr-1(ok401) and arr-1(ok401) animals expressing ARR-1 under the regulation of the sra-6 promoter (Fig. 3, B and C), which drives ARR-1 expression to OCTR-1-expressing neurons ASH and ASI. Neither did the expression of ARR-1 further enhance the up-regulation of abu genes in arr-1(ok401) animals (Fig. 3D). These results suggest that there may be another GPCR in ASH and ASI neurons whose signaling promotes immune homeostasis.

Another GPCR, NPR-1, which is related to mammalian neuropeptide Y receptors, functions in a neural circuit involving AQR, PQR, and URX sensory neurons to enhance immune responses against P. aeruginosa (12). If ARR-1 were responsible for blocking NPR-1 signaling in the nervous system, we would expect npr-1 mutation or expression of ARR-1 in NPR-1-expressing neurons to rescue the ERP phenotype of arr-1(ok401) animals. Indeed, the ERP phenotype of arr-1(ok401) was par-

FIGURE 3. The ERP phenotype of arr-1(ok401) animals is independent of OCTR-1 in ASH and ASI neurons. A, WT, arr-1(ok401), octr-1(ok371), and arr-1(ok401);octr-1(ok371) animals were exposed to P. aeruginosa and scored for survival. B, WT, arr-1(ok401), and arr-1(ok401);pVF4(rof-6[6su1066]) (AY105) animals were exposed to P. aeruginosa and scored for survival. C, WT, arr-1(ok401), and AY105 animals were exposed to P. aeruginosa/GFP for 24 h, and cfu were quantified. D, histogram shows qRT-PCR analysis of abu genes in WT, arr-1(ok401), and AY105 animals exposed to P. aeruginosa for 4 h. A, WT versus octr-1(ok371) (p < 0.0001), arr-1(ok401) versus arr-1(ok401);octr-1(ok371) (p = 0.0066), octr-1(ok371) versus arr-1(ok401);octr-1(ok371) (p < 0.0001). B, representative assays of at least three independent experiments show WT versus AY105 (p < 0.0001), arr-1(ok401) versus AY105 (p = 0.0925). Shown are representative assays of at least three independent experiments; n = 100 animals/strain. C shows mean ± S.E. (error bars); n = 4 independent experiments. D shows mean ± S.E.; n = 3 independent experiments.

FIGURE 2. ARR-1 functions in the nervous system to regulate innate immunity. A, wild type (WT), arr-1(ok401), and arr-1(ok401)pVS3[par-3;arr-1::gfp];pRF4[rol-6[6su1066]] (AY103) animals exposed to P. aeruginosa and scored for survival. B, WT, arr-1(ok401), and AY103 animals exposed to P. aeruginosa/GFP for 24 h, and the cfu were quantified. C, confocal image of AY103 animals expressing ARR-1::GFP in the nervous system. A, WT versus arr-1(ok401) (p < 0.0001), WT versus AY103 (p = 0.7384), arr-1(ok401) versus AY103 (p < 0.0001), D, WT versus AY104 (p = 0.4647), arr-1(ok401) versus AY104 (p < 0.0001). A and D, representative assays of at least three independent experiments; n = 100–120 animals/strain. B and E, mean ± S.E. (error bars). **, p < 0.005; ***, p < 0.0005; n = 4 independent experiments.

response) as they are activated in xbp-1 mutant animals when ER stress is induced (7, 28). They are also up-regulated in response to P. aeruginosa infection and strongly expressed in nonneuronal tissues such as the pharynx and the intestine (7, 28), primary interfaces between host cells involved in immune responses and bacterial pathogens. We found that five abu genes were up-regulated in arr-1 (ok401) animals when exposed to P. aeruginosa. The up-regulation of abu genes was rescued by ARR-1 expression in the nervous system (supplemental Fig. S1), indicating that neural ARR-1 regulates this noncanonical UPR pathway.
extended lifespan of more important role in the regulation of immunity against bac-
tast, we found that suppressed the ERP phenotype of notype of
ADF sensory neurons control DAF-16 activation (29), we stud-
ARR-1 regulates DAF-16-mediated lifespan extension (27) and
Both Pathogen Resistance and Lifespan Extension
arr-1(ok401)
expression to ADF neurons, slightly rescued the extended lifes-
ated by arr-1(ok401)
expression of ARR-1 in the aforementioned neurons (Fig. 4
FIGURE 4. NPR-1 partially controls innate immunity through ARR-1 sig-
neurons, as these neurons have been
in the control of not only temperature sensation and
regulation might control longevity and immune response by act-
that although ARR-1 activity driven to AFD neurons by the
gcy-8 promoter had no effect on the extended lifespan of arr-1(ok401)
arr-1(ok401)
D
C
B
A

FIGURE 5. ARR-1 signaling in ADF neurons suppresses innate immunity
and longevity. A, WT, arr-1(ok401), and arr-1(ok401)pVS7(pshr-142::arr-
1::gfp)::psRF4(rol-6(su1006)) (AY107) animals were scored for lifespan. B, WT,
arr-1(ok401), and AY107 animals were exposed to P. aeruginosa and scored for survival.
AQR, PQR, and URX neurons only plays a
regulation of abu genes in WT, arr-1(ok401), and AY107 animals exposed to P. aeruginosa for 4 h, A, WT versus
arr-1(ok401)pshr-1(ad609) (p = 0.001), WT versus
arr-1(ok401)pshr-1(ad609) (p < 0.0001), arr-1(ok401) versus
arr-1(ok401) (p < 0.0001), B, WT versus
arr-1(ok401) versus
AY106 (p < 0.0001). A and B, representative assays of at least three independent
expression in AFD neurons also partially rescued the enhanced
animals (Fig. 6,
DISCUSSION
We sought to investigate the role of GPCR signaling in the entire nervous system in the regulation of immune response against bacterial infections taking advantage of a unique component of GPCR signaling, the adaptor protein ARR-1. Our findings suggest a broad role for neuronal signaling of ARR-1 in the control of organismal homeostasis. Neural expression of ARR-1 rescued both the extended lifespan and ERP phenotype of arr-1(ok401) animals (Fig. 2D and supplemental Fig. S3), indicating that neuronal ARR-1 controls both of these pro-
tially rescued by npr-1 mutation and by expression of ARR-1 under the control of the gcy-32 promoter, which drives its expression to AQR, PQR, and URX neurons (Fig. 4, A and B).
neurons expressed in AFD Thermosensory Neurons—We hypothesized that ARR-1 signaling might control longevity and immune response by act-
neurons plays a crucial role in the control of immune homeostasis during bacterial infections.
Arr-1 Signaling in ADF Thermosensory Neurons Regulates Both Pathogen Resistance and Lifespan Extension—Because ARR-1 regulates DAF-16-mediated lifespan extension (27) and ADF sensory neurons control DAF-16 activation (29), we studied whether ARR-1 functions in ADF neurons to control either the lifespan extension or the ERP phenotype of arr-1(ok401) animals. We found that although ARR-1 expression under the regulation of the srh-142 promoter, which drives ARR-1 expression to ADF neurons, slightly rescued the extended lifespan of arr-1(ok401) animals (Fig. 5A), it rescued the ERP phenotype of arr-1(ok401) animals more strongly (Fig. 5B). In contrast, we found that daf-16 mutation fully suppressed the extended lifespan of arr-1(ok401) animals (Fig. 1E) and slightly suppressed the ERP phenotype of arr-1(ok401) animals (Fig. 1F). Thus, ARR-1 signaling in ADF neurons appears to play a more important role in the regulation of immunity against bacterial infections than in the control of lifespan. ARR-1 expres-
ion in ADF neurons also partially rescued the enhanced expression of abu genes and reduced bacterial accumulation of arr-1(ok401) animals (Fig. 5, C and D), further supporting the function of ARR-1 signaling in ADF neurons to control immune homeostasis.
neurons involved in thermosensory AFD neurons, we found that npr-1 regulates the expression of abu genes and reduced bacterial accumulation of arr-1(ok401) animals (Fig. 5, C and D), further supporting the function of ARR-1 signaling in ADF neurons to control immune homeostasis.
neurons involved in thermosensory AFD neurons, we found that npr-1 regulates the expression of abu genes and reduced bacterial accumulation of arr-1(ok401) animals (Fig. 5, C and D), further supporting the function of ARR-1 signaling in ADF neurons to control immune homeostasis.
Neural Arrestin-1 Regulates Innate Immunity

FIGURE 6. ARR-1 signaling in AFD neurons suppresses innate immunity. A, WT, arr-1(ok401), and arr-1(ok401);pVF57[parh-142;arr-1:gfp;pRF4(rol-6su1000)](AY108) animals were scored for lifespan. B, WT, arr-1(ok401), and AY108 animals were exposed to P. aeruginosa and scored for survival. C, histogram shows qRT-PCR analysis of abu genes in WT, arr-1(ok401), and AY108 animals exposed to P. aeruginosa for 4 h. D, WT, arr-1(ok401), and AY108 animals were exposed to P. aeruginosa/GFP for 24 h, and the cfu were quantified. A, WT versus AY108 (p < 0.0001), arr-1(ok401) versus AY108 (p = 0.3224); B, WT versus AY108 (p = 0.0021), arr-1(ok401) versus AY108 (p < 0.0001). A and B, representative assays of at least three independent experiments; n = 100 animals/strain. C, mean ± S.E. (error bars); n = 3 independent experiments. D, mean ± S.E.; n = 4 independent experiments. *, p < 0.05; **, p < 0.005.

that GPCR signaling and thermosensation in these cells may play an important role in response to pathogen infections. Fever is an ancient immune mechanism used by metazoans in response to microbial infections, and warm temperatures activate a conserved pathway involving HSF-1 that helps C. elegans fight bacterial infections (33). Whereas only homeotherms are capable of internally increasing the body temperature, both homeotherms and poikilotherms exhibit warmth-seeking behavior when infected. Our results raise the interesting possibility that AFD neurons may participate in circuits that integrate inflammatory cues and thermosensation to fight infections.

In summary, our results indicate that the C. elegans nervous system utilizes a network of sensory neurons that controls longevity and a stress-induced pathway that plays a key role in cellular homeostasis during responses against bacterial infections. Whereas ARR-1 signaling in both AFD and ADF neurons controls immunity, it only partially controls longevity through ADF neurons. The identification of GPCRs in these cells capable of sensing pathogens or the damage caused by infections or aging is an important area for further study.

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