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Interleukin-17: Why the Worms Squirm

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IL-17 is a cytokine known primarily for its role in inflammation. In a recent issue of *Nature*, Chen et al. (2017) demonstrate that IL-17 plays a neuromodulatory role in *Caenorhabditis elegans* by acting directly on neurons to amplify neuronal responses to stimuli and produce changes in animal behavior.

Interleukin-17 (IL-17) is a pro-inflammatory cytokine that in mammals is involved both in host defense and in pathological inflammatory conditions such as autoimmune diseases (Gaffen et al., 2014). An evolutionarily ancient molecule that is more structurally similar to nerve growth factor than to most typical cytokines (Kang and Malhotra, 2015), IL-17 might also play a role in neuronal development and in neuropsychiatric and neurodevelopmental disorders. A recent clinical trial of an anti-IL-17 receptor (IL-17R) antibody had to be discontinued because of an increased risk of suicide (Schmidt, 2015), implicating IL-17 signaling in complex behavioral outcomes. Additionally, work in our lab has demonstrated that fetal mice exposed to elevated levels of maternal IL-17A, one of six IL-17 isoforms in mammals, are born both with behavioral abnormalities analogous to those observed in humans with autism spectrum disorder and with disordered cortical development in their brains (Choi et al., 2016). Now, Chen et al. (2017) provide convincing evidence that an IL-17-like homolog in *C. elegans* can act directly on neurons under physiological conditions as a neuromodulator to affect neuronal activity and animal behavior.

Unlike neurotransmitters, which convey fast and precise signals directly from pre- to post-synaptic neurons, neuromodulators can travel and act across distances to coordinate networks of cells and even robust behavioral outcomes. For example, oxytocin (OT), which is produced primarily in the supraoptic and paraventricular nuclei of the hypothalamus, has been shown to modulate neuronal activities in multiple brain regions and affect reward and emotional processes and social bonding (Stoop, 2014). Although the neuromodulatory effects of neuropeptides such as OT and the closely related vasopressin have been studied extensively, recent studies have also begun to uncover the underappreciated function of immune molecules as neuromodulators. For example, the inflammatory cytokine IL-18 is necessary for the induction of a stress-induced depression phenotype in mice by acting at the basolateral amygdala (Kim et al., 2016). Furthermore, another inflammatory cytokine, interferon gamma (IFN-γ), has multiple roles in different brain regions: in the hippocampus, it acts selectively to reduce GABAergic inhibition, leading to network hyperexcitability (Zhu et al., 2011), whereas in the prefrontal cortex, it acts on neurons to increase inhibitory GABAergic tone, affecting social behavior in mice (Filiano et al., 2016). The finding by Chen et al. (2017) that an IL-17 homolog plays a neuromodulatory role in *C. elegans* is a valuable addition to our understanding of the effects of inflammatory cytokines on neuronal function.

The nematode *C. elegans* has two general behavior patterns in response to food—foraging either individually or in aggregate—and these differences in behavior are due to variation in the gene *npr-1* (neuropeptide receptor 1), whereby those worms that carry a null mutation exhibit the aggregating behavior (Laurent et al., 2015). Chen et al. (2017) started with these aggregating *npr-1*-null worms and performed a forward genetic screen to generate mutations and look for loss of aggregation. Of the over 800 such poor aggregators, they sequenced the genomes of 583 worms and found that six had mutations in an IL-17R ortholog, which they named *ilcr-1*. Deletion of *ilcr-1* in aggregating *npr-1*-null worms converted them to solitary feeders, and expression of *ilcr-1* cDNA in these worms rescued the aggregating phenotype. Furthermore, although it wasn't found in their original screen, *C. elegans* has a second IL-17R-like protein, which the authors named *ilcr-2*. When knocked out in aggregating animals, it also produced solitary behavior. Next, the authors looked for genes homologous to IL-17 itself and found three, one of which was represented in their original genetic screen. The authors named this gene *ilc-17.1* (IL-17-cytokine-related gene 1), and as with the two receptor genes, they found that they could convert aggregating animals to solitary ones in *npr-1*-null worms. Notably, disrupting more than one of these three genes (*ilcr-1*, *ilcr-2*, and *ilc-17.1*) did not produce a unique or stronger phenotype, suggesting that all are involved in the same pathway.

Chen et al. (2017) next endeavored to demonstrate that *ilcr-1*, *ilcr-2*, *and ilc-17.1* interact physically and in ways similar to IL-17 cytokines and receptors in mammalian cells. By expressing fluorescently tagged versions of ILCR-1 and ILCR-2 in HEK293T cell lines, the authors demonstrated both that the proteins co-localized on the plasma membrane and that they precipitated together in a co-immunoprecipitation (coIP) experiment. Furthermore, by adding fluorescently tagged ILC-17.1, they found that it strongly interacted with the surfaces of cells expressing both ILCR-1 and ILCR-2. These findings suggest that, like in mammals (where IL-17-induced signal often requires a heterodimeric complex of two different IL-17R subunits), ILC-17.1 signals via an ILCR-1-ILCR-2 heterodimer.

In order to further examine the role of this IL-17-like signaling pathway, Chen et al. (2017) took advantage of the fact that aggregating *C. elegans* respond to

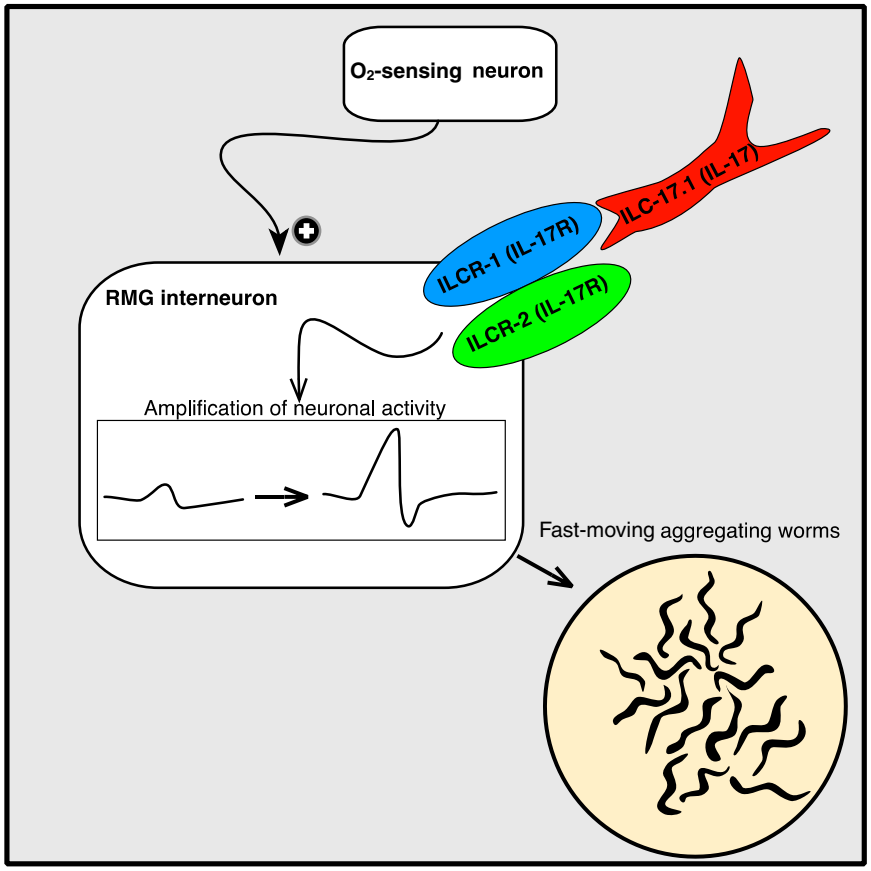

Figure 1. The IL-17 Ortholog ILC-17.1 Modulates Neuronal Activity in RMG Interneurons
Chen et al. (2017) demonstrate that the *C. elegans* IL-17 ortholog ILC-17.1 signals via a heterodimer of two IL-17R orthologs (ILCR-1 and ILCR-2) on RMG interneurons to produce increased neuronal activity in response to input from O_2-sensing neurons exposed to 21% O_2. RMG interneuron activation in *npr-1*-null worms leads to increased velocity of movement and to increased aggregation behavior.

an increase in oxygen (O_2) concentration from 7% to 21% by greatly increasing their speed of travel. This response is mediated by RMG interneurons, which produce rapid forward movement when activated by specific O_2-sensing URX neurons (Laurent et al., 2015). When present, NPR-1 inhibits this forward motion by decreasing the output of RMG interneurons (Laurent et al., 2015). Chen et al. (2017) found that whereas aggregating *npr*-1-null worms maintained their increased speed in response to elevated O_2, *npr*-1-null worms carrying mutations in any of the three identified IL-17-associated genes only transiently increased their speed and returned to baseline after 2 hr of high O_2 exposure. Although ILCR-1 and ILCR-2 were expressed in RMG and other neurons, the escape response to 21% O_2 was restored robustly only when the gene was ectopically expressed in RMG neurons, suggesting that ILCR coreceptors act predominantly in RMG interneurons to affect aggregation and O_2 response behavior. The authors performed another set of experiments to show that ILC-17.1 is expressed in several types of neurons, including RMG interneurons. Unlike with the ILCRs, expressing ILC-17.1 in multiple cell types recovered the O_2 response phenotype in mutant worms, demonstrating that the source of the protein need not be restricted to RMG interneurons.

To determine the effect of this IL-17-like signaling pathway on RMG interneurons, Chen et al. (2017) measured neuronal activity both by looking at Ca^{2+} responses to 21% O_2 and through expression of a fluorescently tagged neuropeptide reporter. Worms with mutations in the IL-17-like genes had decreased RMG neuronal activity in response to 21% O_2, and the response was rescued by expression of the appropriate gene in RMG neurons. These RMG interneurons are also involved in responses to pheromones. The authors found that *ilcr-17.1* mutants were repelled by a mix of ascarosides that would be expected to attract the animals, but they still responded normally to other chemicals that act through other neurons, such as NaCl. Therefore, mutations in this IL-17-like pathway affect only those behaviors associated with RMG interneurons and do not induce general circuit dysfunction. Importantly, using channelrhodopsin to artificially increase neuronal activities in the RMG neurons of *ilc-17.1* mutants evoked behavioral responses comparable to those in control animals, demonstrating that the main function of ILC-17.1 is to modulate RMG neuronal activity and not downstream signaling pathways (Figure 1).

In gain-of-function experiments, Chen et al. (2017) demonstrated that overexpression of ILC-17.1 intensified responses to 21% O_2 and even caused wild-type non-aggregating worms to aggregate. However, although the excess ILC-17.1 increased RMG neuronal response to 21% O_2, it did not affect neuronal activity at 7% O_2, suggesting that ILC-17.1 overexpression did not depolarize RMG neurons per se but rather amplified neuronal responses to additional inputs. Furthermore, ILC-17.1 specifically enhanced RMG activity, given that it did not affect that of O_2-sensing URX neurons.

Finally, Chen et al. (2017) looked to their genetic screen for potential hits in the downstream ILC-17.1 signaling pathway and found several in three genes that phenocopied mutants of *ilcr-1*, *ilcr-2*, and *ilc-17.1* when mutated. Two of these genes, *actl-1* and *nfki-1*, are homologs of genes coding for proteins that act downstream of IL-17R in mammals: ACT1 and the atypical NF-κB inhibitors NFKBID and NFKBIZ/IκBζ, respectively. The third gene, *pik-1*, encodes a homolog of IRAK (interleukin-1-receptor-associated kinase), a protein associated with toll-like receptor signaling in mammals. Using coIP assays, the authors demonstrated that, like the mammalian protein, *C. elegans* ACTL-1 interacts with IL-17Rs but that, unlike in mammals, *C. elegans* PIK-1 interacts with ACTL-1, thereby making this IL-17-like signaling

pathway a hybrid mix of the mammalian toll-like receptor and IL-17R pathways. Mutations among these three genes or in combination with *ilc-17.1* did not produce enhanced phenotypes, providing evidence that all of these proteins most likely function in the same genetic pathway.

This work by Chen et al. (2017), built on robust and unbiased genetic screens, provides convincing evidence that this ancient and unusual cytokine might act directly on neurons to modulate neuronal activity and behavioral output, but it leaves a few critical questions open for future study. Broadly speaking, although the authors briefly hypothesized that this IL-17-like signaling pathway could be involved in pathogen avoidance, they did not further explore a possible primitive immune function for ILC-17.1. Moreover, it is not clear how enhanced IL-17-like signaling in RMG interneurons leads to increased neuronal activation. Lastly, it will be informative to study how ILC-17.1 expression is controlled; is it constitutively expressed or induced upon exposure to certain environmental cues or pathogens? Regardless, this work by Chen et al. (2017) is both thought provoking and exciting and adds another stepping stone toward understanding the roles played by immune effector molecules in the human brain in both normal and pathological conditions.

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