Neuronal modulation of the immune response

The nervous and immune systems respond in distinct manners to diverse signals. Crosstalk between these systems has been known: microbial infections can result in inflammation of nervous tissue and lead to conditions such as encephalitis and meningitis. Also, the immune response causes damage to nervous tissue during autoimmune diseases: extensive axonal damage is observed in multiple sclerosis due to demyelination and infiltration by leukocytes (Mix *et al* 2007). To study the roles of immune molecules, researchers often use a mouse model of multiple sclerosis known as experimental autoimmune encephalomyelitis in which disease is induced upon injection of myelin oligodendrocyte glycoprotein in Complete Freund's adjuvant. Here, inflammatory cytokines, e.g. Interferon-γ (Tran *et al* 2000) and Tumour necrosis Factor-*α* (Liu *et al* 1998), or Toll-like receptors (TLR)-4 or TLR-9, which detect microbial constituents (Marta *et al* 2008), play regulatory roles to reduce disease severity. In cases like these, it is not too surprising that inflammatory immune molecules affect nervous tissues. On the other hand, proper understanding is lacking on how neuronal products modulate immune reactions, especially with respect to anti-pathogen responses.

Two recent papers (Kawli and Tan 2008; Evans *et al* 2008) shed new light on the modulation of the immune response by neuronal products. The authors used the soil nematode *Caenorhabditis elegans* to investigate the response to bacterial infections. There are some distinguishing features of this system (Gravato-Nobre and Hodgkin 2005): (i) *C. elegans* displays an inducible innate immune response as is present in non-vertebrates. It is perhaps appropriate to mention that vertebrates display both innate and adaptive immune responses. The innate response is rapid but non-specific whereas the adaptive response is slower, specific and demonstrates memory. Specificity during the adaptive response is due to the activation and proliferation of clonal B cells or T cells via specific B cell or T cell receptors. Non-vertebrates do not demonstrate this response as the recombination activated genes (RAG) and diverse B cell and T cell antigen receptors are present only in vertebrates. (ii) The transparent body of *C. elegans* allows visualization of the infection processes. (iii) *C. elegans* is easily amenable to RNA interference (RNAi)-based gene silencing. (iv) *C. elegans* mutants with loss in neuronal function survive and do not demonstrate any serious effects. Therefore, it is relatively easy to identify neuronal genes that modulate immunity. (v) Finally, studies in *Drosophila melanogaster* have shown that the TOLL (directed against fungal and Gram positive bacteria) and IMD (predominantly against Gram negative bacteria) pathways regulate the production of anti-microbial responses via NF-κB-like transcription factors (Ferrandon *et al* 2007). However, *C. elegans* lacks NF-κB and is therefore a convenient model to identify the roles of non-NF-κB mediators during the immune response.

C. elegans feeds on bacteria and is usually propagated on a lawn of *Escherichia coli*. After feeding, bacteria are pumped into the pharynx where a grinder-like organ disrupts most bacteria; viable *Escherichia coli* are usually not found in the intestine. However, upon feeding pathogenic bacteria, the nematode may die. In that case, colony forming units (CFU) of bacteria can be recovered from the intestine. The authors observed that infection with *P. aeruginosa* (but not other bacteria, e.g. *Salmonella enterica* or *Enterococcus faecalis*) suppressed the production of immune effector molecules. Consequently, more CFUs and a higher mortality was observed in *C. elegans* upon infection with *P. aeruginosa.* Surprisingly, neuronal mutants defective in neurosecretion or neuropeptide processing showed an increase in survival relative to the wild type upon infection. Concomitantly, mutants that caused greater neurosecretion were more sensitive to infection. Importantly, these mutants did not show a generalized sensitivity to other stressful conditions, e.g. high osmolarity or cadmium.

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Further studies demonstrated that an insulin-like peptide (INS)-7 was induced and secreted by neuronal cells upon *P. aeruginosa* infection. INS-7 binds to DAF-2, a transmembrane tyrosine kinase insulin receptor, and enhanced signalling by this receptor leads to cytosolic localization of intestinal DAF-16, a member of the forkhead transcription factor family. This relocalization (from the nucleus to the cytosol) leads to reduced transcriptional activation of several immune response genes: *lys-7* (encoding a lysozyme-like protein), *spp-1* (encoding a saponin-like protein), *thn-2* (encoding a member of the thaumatin family of plant anti-fungal proteins) etc. The tissue-specificity of gene expression i.e. neuronal INS-7 and intestinal DAF-16, is important, because the intestine is the target of *P. aeruginosa* infection and the site of expression of host defense genes. Clearly, a correlation exists between the secretion of INS-7, the DAF-2 signalling pathway and the cytosolic location of DAF-16, which leads to a lowered immune response and greater susceptibility to infection by *P. aeruginosa* (figure 1).

These studies, demonstrating a sensitive mechanism to detect and respond to pathogenic bacteria, are important and raise several questions: What are the roles of other neurosecretory peptides and their receptors in immune modulation? Perhaps, the identification of INS-7 is the tip of an iceberg and there are other players and pathways that need to be studied. Also, DAF-2 and DAF-16 are important in other

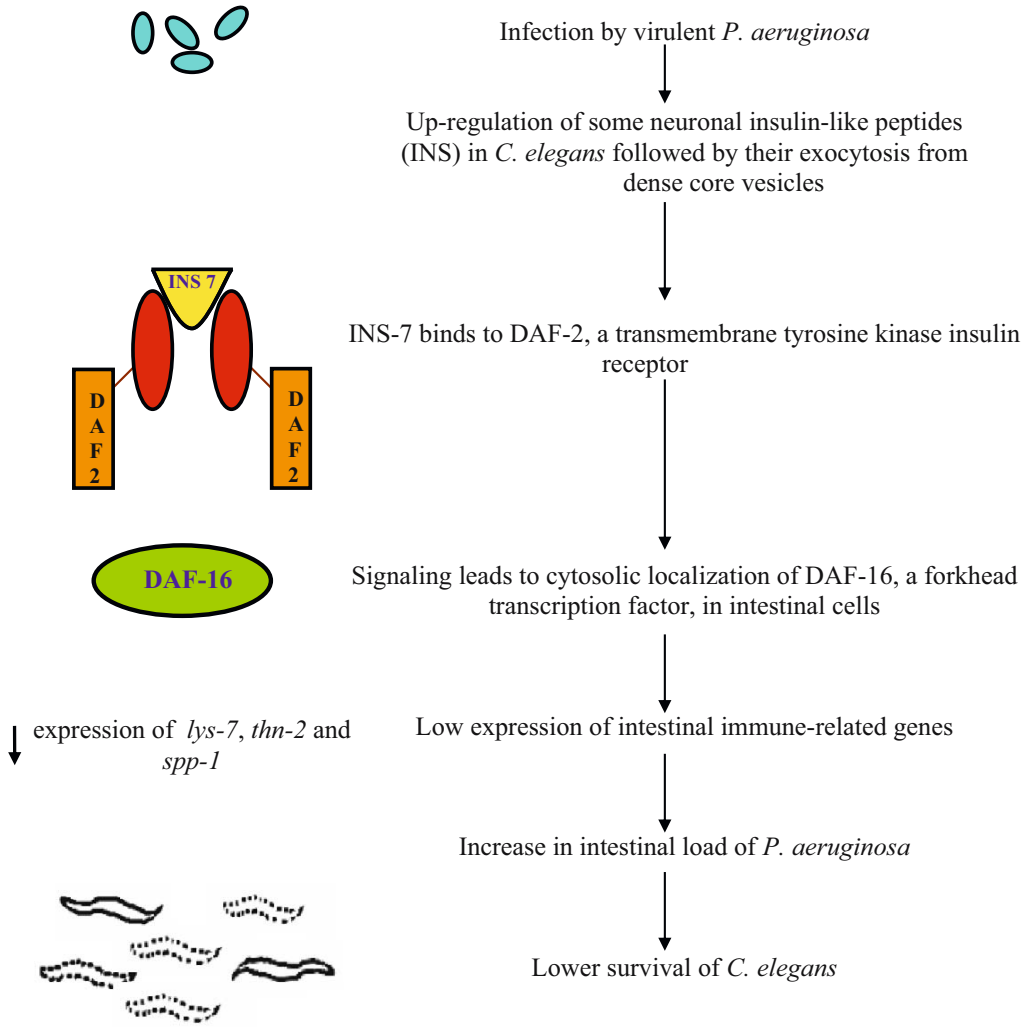

Figure 1. Flow chart of events occurring upon infection of *C. elegans* with virulent *P. aeruginosa.* The outcome is the activation of an insulin-like signalling pathway followed by a reduction in the immune response.

cellular functions. *C. elegans* lacking *daf-2* are long-lived and resistant to oxidative stress whereas those lacking *daf-16* are short lived and sensitive to oxidative stress (Ogg *et al* 1997; Garsin *et al* 2007). The transcriptional targets of DAF-16 have been studied (Oh *et al* 2006) and it may be useful to identify those directly involved in immune defense and functionally evaluate their roles during infection.

What are the implications of these studies for higher organisms? With respect to innate immune responses, it is known that Toll-like receptors enhance inflammatory responses and modulate insulin signalling during obesity (Shi *et al* 2006; Kim *et al* 2007). However, the role of insulin signalling in directly modulating immune responses is not well known. Interestingly, there are several insulin-like peptides in mice and humans which bind to G protein coupled receptors and play diverse roles, e.g. in collagen turnover, fertility, pregnancy, etc. (van der Westhuizen *et al* 2008). A closer look is now required to evaluate their effects on immune cell gene expression and function in vertebrates.

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