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Extra View

Heat Shock and Genetic Activation of HSF-1 Enhance Immunity to Bacteria

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ABSTRACT

The relationship between fever and microbial infections has been known for a number of years, as well as several key mediators involved in its elicitation. However, the mechanisms by which fever confers protection to infected hosts are less clear. The nematode *Caenorhabditis elegans*, which has been extensively used in recent years to study microbial infections and innate immune responses, has recently been used to study the effect of increased temperature in immunity. Upon heat shock exposure, nematodes become more resistant to *Pseudomonas aeruginosa* and the enhanced resistance to the pathogen requires heat shock transcription factor 1 (HSF-1) and a system of small and 90 kDa heat shock proteins (HSPs). Experiments using additional Gram negative and Gram positive pathogens show that HSF-1 is part of a multipathogen defense pathway. In addition, *C. elegans* innate immunity can be activated enhancing HSF-1 activity by directly overexpressing HSF-1 or by overexpressing DAF-16, which is a forkhead transcription factor that acts upstream HSF-1 in aging and immunity. Blocking the inhibitory signal of the DAF-2 insulin like receptor, which acts upstream DAF-16, also results in an enhanced HSF-1 dependent immunity. In addition, mutations that affect DAF-21, *C. elegans* homologue of Hsp90 which forms an inhibitory complex with HSF-1, appear to boost immunity by activating the HSF-1 pathway. The role of the HSF-1 pathway in innate immunity and immunosenescence is discussed.

INTRODUCTION

Fever is an ancient immune mechanism used by metazoans in response to microbial infections. It has been very well documented that injections of lipopolysaccharide (LPS), which is a major component of the outer membrane of all Gram negative bacteria, elicit both warmth seeking behavior and fever in rats.[1-5] A number of studies have reported that besides homeotherms, such as rats that are capable of internally increasing the body temperature, poikilothermic vertebrates and invertebrates develop behavioral fever in response to injections of LPS.[6] In mammals, where the molecular mechanisms of fever initiation have been very well studied, it is known that endogenous pyrogens stimulate the production of prostaglandins which are the main mediators of increased temperature.[7-13] Indeed, the extensively used nonsteroidal anti inflammatory drugs reduce fever by targeting prostaglandin synthesis.

Although several studies have been conducted to understand the mechanism of fever elicitation and to develop antipyretic therapeutics, the mechanism by which fever exerts its beneficial role has not been studied in detail. It is believed that fever may benefit infected hosts by: (1) affecting the viability of certain invading microbial pathogens; (2) increasing host resistance to chemical stresses generated by the infection inducing heat shock proteins (HSPs), (3) stimulating pathogens to generate HSPs, which can activate the host immune system, and (4) regulating host defenses.[14] We have used the genetically tractable organism *C. elegans* to study the mechanism by which increased temperatures activate the innate immune system.[15] We found that a conserved heat shock pathway that requires heat shock factor 1 (HSF-1) is important for *C. elegans* immunity against bacterial pathogens and that both small and 90 kDa heat shock proteins (HSPs) activated in an HSF-1 dependent manner are the effectors responsible for immune protection. Our results also showed that the HSF-1 pathway regulates immunity independently of p38 MAPK and that it interacts with the DAF 2/DAF-16 pathway which regulates aging and immunity in nematodes. Here we discuss the role of the HSF-1 pathway in innate immunity and immunosenescence, and we provide additional evidence indicating that genetic activation of the pathway protects against bacterial infections.

INNATE IMMUNE ACTIVATION BY HEAT SHOCK OCCURS INDEPENDENTLY OF p38 MAPK

In a study designed to identify components of the *C. elegans* immune system required for proper response to bacterial infection, Kim et al.[16] identified two genes which encode components of a conserved p38 MAPK signaling pathway. The mutations identified corresponded to *C. elegans* genes encoding MAPKKK/NSY 1 and MAPKK/SEK 1, which are required for the activation of *C. elegans* MAPKKK-PMK-1, homologue of mammalian p38. Subsequent studies showed that the p38/PMK-1-MAPK pathway is required for *Salmonella enterica* elicited programmed cell death in the *C. elegans* germline[17] and for defense to *Bacillus thuringiensis* toxin Cry5B.[18] Additional studies also showed that TIR 1 is required for the activation of PMK-1.[19]

These results suggest that, as p38 in mammals, PMK-1 plays a key role in mediating *C. elegans* immune functions. Since p38 MAPK signaling is known to mediate stress responses and is activated by heat shock in mammals, it was studied whether the pathway was also required for heat shock mediated activation of immunity in *C. elegans*.[15] Heat shocked mutants in the p38/PMK-1 pathway were more resistant to *P. aeruginosa* than untreated animals indicating that heat shock mediated protection is independent of p38/PMK-1 MAPK activation and that it may require other heat shock induced proteins.

GENETIC ACTIVATION OF THE HEAT SHOCK PATHWAY BOOSTS IMMUNITY TO BACTERIA

It was shown that *C. elegans* HSF-1 is required for heat shock induced immunity and constitutive immunity, as loss of HSF-1 through mutation or RNA interference (RNAi) ablation of expression results in an increased susceptibility to *P. aeruginosa* of both heat shock and control animals.[15] Additional experiments show that the HSF-1 defense pathway requires inducible heat shock proteins including those from the Hsp90 and small HSP families. DAF-16, which is a forkhead transcription factor positively regulated by heat shock[20] and negatively regulated by DAF-2,[21] was found to be required for the activation of HSF-1.

If HSF-1 is required for proper immunity, a prediction would be that animals expressing higher levels of HSF-1 would be more resistant to pathogens. Several genetic approaches were taken to demonstrate that HSF-1 activation increases resistance to bacterial pathogens. It was found that transgenic animals overexpressing HSF-1 were more resistant to *P. aeruginosa* as well as animals overexpressing DAF-16, which increases HSF-1 activity.[15] Lack of DAF-16 and HSF-1 inhibition by mutation in DAF-2, which encodes a negative regulator of aging and immunity, was also found to increase immunity to Gram positive and Gram negative bacteria.[15] These results show that genetic activation of the HSF-1 pathway boosts immunity to bacterial pathogens and they open the possibility of using drugs capable of activating HSF-1 to treat infectious diseases and immunodeficiencies.

MUTATION IN HSP90 ENHANCES IMMUNITY VIA HSF-1

Since HSF 1 is maintained in the inactive cytosolic state as a monomer bound to the Hsp90 chaperone,[22] perturbation of the Hsp90 HSF-1 complex using Hsp90 inhibitors, such as geldanamycin, or stresses results in HSF-1 release and subsequent activation, which can be readily measured in terms of Hsp90 induction. Because *daf-21(p673)* animals harbor a temperature sensitive mutation in a highly conserved residue of the DAF-21/Hsp90 encoding region involved in protein binding[23] and similar mutations are known to increase HSF activity,[24] we postulated that at the restrictive temperature the Hsp90/HSF-1 complex could be disrupted resulting in the activation of HSF-1.

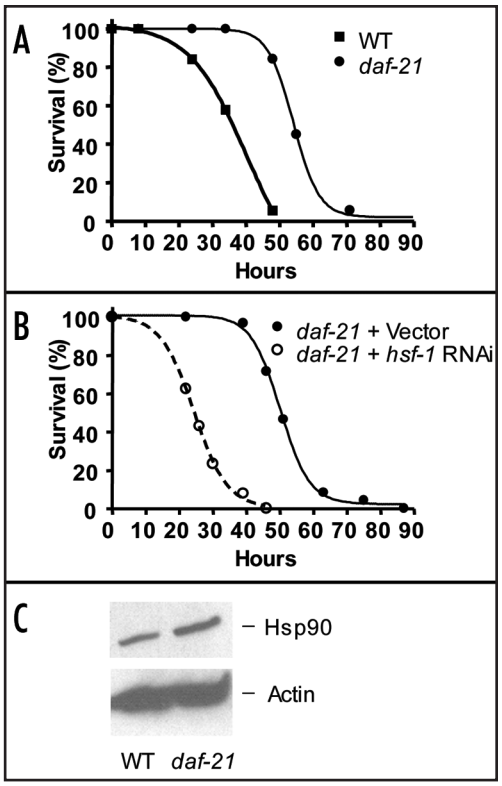

Figure 1. Mutations in daf 21/hsp90 enhanced immunity in an HSF 1 dependent manner. (A) *daf-21(p673)* young adult animals are more resistant to the human opportunistic pathogen *P. aeruginosa* than wild type animals. (B) RNAi of *hsf-1* abolishes the resistance of the daf-21(p673) mutant to *P. aeruginosa*, indicating that the increased resistance of *daf-21(p673)* mutants is due to higher HSF-1 activity. (C) The immunological detection of Hsp90 in wild type and *daf-21(p673)* animals indicates that *daf-21(p673)* nematodes exhibit constitutively higher levels of Hsp90 compared to wild type animals.

As shown in Figure 1C, *daf-21(p673)* animals exhibit constitutively higher levels of Hsp90 compared to wild type animals. *daf-21(p673)* mutants were significantly more resistant to *P. aeruginosa* infection than wild type animals (Fig. 1A), as expected based on the high levels of HSF-1 activity they express (Fig. 1C). Because *daf-21* mutants, when grown at 25°C, are dauer constitutive and may be more resistant to general stresses and exhibit an increased lifespan, the animals were grown at 15°C until they reached adulthood and then they were exposed to *P. aeruginosa* at 20°C (not shown) or 25°C (Fig. 1A). Under these conditions, *daf-21* animals are not dauer constitutive and should not have an increased lifespan. Also, Morley et al. demonstrated that *daf-21* RNAi animals exhibit wild type lifespan.[25] Figure 1B shows that RNAi of HSF-1 abolishes the resistance of the *daf-21(p673)* mutant to *P. aeruginosa*, providing additional evidence that the mutation in *daf-21* animals may affect the Hsp90 HSF-1 complex and result in the activation of HSF-1.

At this point, it is unknown whether HSF-1 and HSPs are part of a constitutive immune response or whether this response is activated by pathogens. Initial attempts to address whether pathogens activate HSF-1 have proven inconclusive. This is not surprising given the

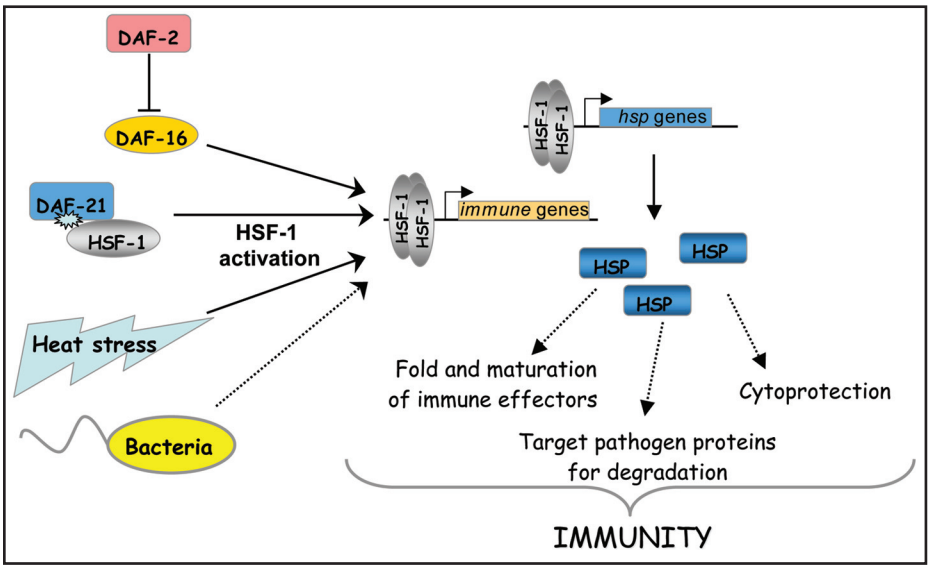

Figure 2. Model showing the regulation of *C. elegans* immunity by the HSF-1 genetic pathway. HSF-1 activated by heat shock, destabilization of the Hsp90-HSF complex, DAF-2, or bacterial pathogens enhances innate immunity. HSF-1 mediates protection via induction of Hsp90 and small HSPs. HSF-1 and the chaperone system may directly or indirectly regulate the expression of innate immunity genes. The system of chaperones may be required for cytoprotection, recognition and degradation of bacterial virulence factors, or proper folding of effector molecules of the immune system.

wide number of potential roles in the organism that could be played by HSF-1. Pathogens may lead to HSF-1 activation in only a subset of *C. elegans* cells that cannot be detected in whole animal lysates. Using in situ hybridization to detect daf-21 mRNA and a monoclonal antibody against DAF-21, it has been reported that daf 21 is expressed all over the body in response to heat stress.[26] This indicates that somatic cells are capable of expressing the protein upon the proper stimuli. The identity of such cells and whether they respond to pathogen attacks activating the HSF-1 remains to be elucidated.

ROLE OF HSF-1 IN IMMUNOSENESCENCE

Decline in immune response with age, termed immunosenescence, is a conserved process that has been observed in vertebrates as well as invertebrates. Immunosenescence makes the elderly more prone to infection due to deterioration or dysregulation of both adaptive and innate immunity responses.[27,28] Decline in HSF-1 activity with age has also been observed in mammals. Although the level of HSF-1 protein remains the same, its DNA binding activity declines with age and also leads to decline in Hsp70 transcription.[29-32] Immunosenescence also appears to take place in *C. elegans* since older nematodes are more susceptible to *P. aeruginosa*, *S. enterica*, *Burkholderia cepacia*, or *Yersinia tuberculosis* than younger nematodes.[33] In addition, it has been reported that *C. elegans* larvae are resistant to *S. marcescens* while older nematodes are susceptible to the same infection.[34]

As in mammals, aging in nematodes is accompanied by a declined or deregulated HSF-1 activity, measured as heat inducibility of HSF-1 dependent chaperone Hsp16.2,[35] suggesting that the immunosenescence phenotype of *C. elegans* may be due to loss of HSF-1 activity. It would be interesting to examine the extent to which HSF-1 activity regulates immunosenescence observed in both nematodes and mammals. *C. elegans* provides the opportunity to further dissect this pathway in terms of regulators and effectors by using excellent genetic tools available for the organism.

POSSIBLE ROLES OF THE HSF-1 PATHWAY IN IMMUNITY

The model shown in Figure 2 outlines our understanding of how HSF-1 might regulate *C. elegans* immunity to bacterial pathogens. HSF-1 can be activated by heat shock, DAF-16, and by destabilization of the HSF Hsp90 complex. Although, HSF-1 does not appear to be activated at the whole animal level by bacterial pathogens, it is possible that bacterial pathogens activate it in a subset of cells crucial to immune response. Future studies to identify the anatomical foci of action of HSF-1 by analyzing genetic mosaics, animals in which different cells have different genotypes, should allow to draw conclusions about the primary anatomical foci of action of HSF-1 and related genes. The use of transcription profiling analysis using microarrays to increase the number of target genes that can be monitored will help to address whether HSF-1 is part of a constitutive immune response or whether it is activated by pathogens; and it will also help identify the mechanism of such activation.

HSF-1 and a chaperone system, consisting of at least 90 kDa and small HSPs, may regulate immunity in a number of ways. HSF-1 and the chaperones may directly or indirectly regulate the expression of innate immunity genes. HSPs may be required for cytoprotection from cell injury due to pathogen proliferation and for proper folding of proteins involved in immune response, including effector molecules. Hsp90 has been shown to act as a chaperone for NODs[36] and to be required for the preactivation and signaling competence of Arabidopsis immune effectors known as resistance (R) proteins.[37,38] It is likely that *C. elegans* homologues also require one or more HSPs for adequate folding and maturation. Another possibility is that the chaperone system may be required for recognition and degradation of bacterial virulence factors that enter the host cells. HSPs can recognize bacterial proteins and target them for degradation. A good candidate for such a function is Hsp70, which has a wide range of substrates. There is evidence indicating that Hsp70 and its cochaperone CHIP, which also possesses E3 ubiquitin ligase, target Hsp70 substrates for degradation.[39] Although RNAi ablation of Hsp70 encoding genes does not increase *C. elegans* susceptibility to pathogens,[15] the possibility of redundancy or incomplete RNAi seen has not been ruled out. Future studies will be required to distinguish among these possibilities.

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