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The Jun Homolog Jra Mediates Toxin Response In Drosophila Melanogaster.

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Abstract

Insects that ingest microbial pathogens are also exposed to their toxins. The sensitivity of insects to ingested toxins of human pathogens and the potential mechanism of toxin resistance has not been thoroughly studied. We tested the survival of *Drosophila melanogaster* orally fed with exotoxins and endotoxins of ten human bacterial pathogens. We discovered that only a few toxins adversely affect fly survival, and that most toxins either do not affect or paradoxically extend insect survival (hormetic effect) at the dosages tested. We found that in *Drosophila*, Jra, a homolog of stress response transcription factor Jun, mediates a broad-spectrum toxin response, since the survival of Jra mutants was shortened in the presence of most of the tested toxins. This study begins to uncover the mechanism of the response of insects to toxins. It describes how a toxin-induced Jun stress response system helps insects reduce their sensitivity to toxins of human pathogens.

Keywords: toxin; sensitivity; resistance; survival; immunity; *Drosophila melanogaster*

Abbreviations: Jun related antigen (Jra); immune deficiency (Imd); antimicrobial peptides (AMP); mitogen-activated protein kinase (MAPK); Bloomington *Drosophila* stock center (BDSC); wild type (WT); lipopolysaccharide (LPS); adenosine diphosphate (ADP); adenosine triphosphate (ATP); adenosine monophosphate (cAMP); Jun N-terminal Kinase (JNK); Nuclear Factor-κB (NF-κB); absorption, distribution, metabolism, and excretion (ADME); absorption, distribution, metabolism, excretion, and toxicity (ADMET); confidence interval (CI); soluble N-ethylmale-imide-sensitive factor-attachment protein receptors (SNARE); basic leucine zipper (bZIP); deoxyribonucleic acid (DNA); eukaryotic elongation factor (eEF); programmed death-ligand (PD-L); cluster of differentiation (CD).

Introduction

Insects often serve as vectors for pathogens of humans, cattle, and mammalian wildlife. Non-biting insects feeding on the bodily fluids of infected carcasses may subsequently deposit contaminated feces and regurgitations on nearby vegetation and expose grazing herbivores to various pathogens and their toxins (1). Similarly, biting flies and mosquitoes have been shown to acquire pathogens from infected animals and transmit them to other mammals (2). Other biting arthropods, such as ticks, also contribute to pathogen transmission (3-5), raising the question of how these vectors tolerate potentially harmful human pathogens and their toxins. Indeed, the vectoring of toxin-producing human pathogens by insects and other arthropods has been reported for *Bacillus anthracis* (2, 6-10), *Vibrio cholerae* (11, 12), *Escherichia coli* (11, 13), *Pseudomonas aeruginosa* (13), *Corynebacterium diphtheriae* (14-16), *Pasteurella multocida* (17), *Bordetella pertussis* (18), *Clostridium difficile* (19, 20), *Clostridium septicum* (21), and *Salmonella species* (22, 23).

To defend against invading microbes, insects produce antimicrobial peptides (AMP) that target microbial cell walls and employ circulating hematocytes to eliminate microbes via reactive oxygen species damage and phagocytosis (24). Insects utilize two evolutionarily conserved signaling pathways, Toll and the immune deficiency (Imd), to express AMP in response to microbial pathogens. The Toll pathway is activated by lysine (lys)-type peptidoglycan found in cell walls of most Gram-positive bacteria (25). The Imd pathway is mainly activated by lys-derived diaminopimelate (dap)-type peptidoglycans found in cell walls of Gram-negative bacteria, *Bacillus* species, and most *Clostridium* species, and by fungal cell wall components (26-28). In addition

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to AMP and phagocytic responses, microbial pore-forming toxins induce fly intestinal epithelia to undergo a thinning (purging) followed by a rapid recovery of initial thickness, contributing to the maintenance of gut wall integrity during microbial infections (29).

While the innate immune response to microbes is well-known in insects and mammals, the innate immune response to toxins is not well understood. Recent studies with *Staphylococcus* aureus pore-forming α-toxin showed that the bacteria induce the secretion of toxin receptor-bearing exosomes from mammalian cells, which act as toxin decoys by scavenging and preventing toxins from binding to target cells (30). Furthermore, conserved recovery mechanisms allow host cells to repair mechanical damage inflicted by toxins, such as plasma membrane repair by the lipogenic process and clogging and removing toxin pores (31). The intracellular responses to toxins include cytoskeleton remodeling and cell survival pathways such as Mitogen-Activated Protein Kinase (MAPK) pathways (31). Moreover, host cell autophagy of toxin-containing organelles is a central defense mechanism against toxins (31).

Several reports showed that microbial toxins increase the abundance of a stress response transcription factor, Jun, in human cells (32, 33) and the homolog Jun-related antigen, Jra, in *Drosophila* (34, 35). In the absence of bacteria, Jun is activated by *S. aureus* α-toxin and is necessary for the resistance of host cells to this toxin, shown by increased sensitivity to α-toxin in Jun-deficient cells (32). In *Drosophila*, expression of Jra is activated in response to cholera toxins and *E. coli* lipopolysaccharide (LPS) treatments (34-36). These observations suggest that Jun may be essential for activating the response to both endo- and exotoxins of bacterial pathogens and microbial toxins. We hypothesized that Jra is necessary for innate immune response to microbial toxins in *Drosophila*. This study investigated insect sensitivity to several endo- and exotoxins. It began to uncover the mechanism of the response to many toxins in the *D. melanogaster* model of oral intoxication. Our experiments provide further insights into mammals' innate immunity mechanisms against bacterial toxins.

Methods

Chemicals and reagents

Toxins used for *Drosophila* feeding assays (Table 1) were purchased from List Biological Laboratories (Campbell, California). Toxins (product numbers) used for screening in *Drosophila* included: *Bordetella pertussis* toxin (180) and adenylate cyclase toxin (188L), *Clostridium difficile* toxins A (152C) and B (155B), *Clostridium perfringens* ε-toxin (126A), *Clostridium septicum* α-toxin (116L), *Corynebacterium diphtheriae* toxin (150), *Escherichia coli* O111:B4 lipopolysaccharide (201), *Pasteurella multocida* toxin (156), *Salmonella typhimurium* lipopolysaccharide (225), *Salmonella minnesota* R595 lipopolysaccharide (304) and monophosphoryl lipid A (401), and *Vibrio cholerae* cholera toxin (100B).

Drosophila rearing

Oregon-R *Drosophila melanogaster* (Bloomington Drosophila Stock Center (BDSC) stock #2376) fly strain was used to conduct wild-type (WT) experiments. Mutant strains (stock # from BDSC) used in this study were: Jra (7218), Jra (7217), and Jra^{IA109} (3273). Fly strains were kept at 25°C with 12-hour light/dark cycles. All strains were fed a standard cornmeal-molasses-agar fly medium with yeast flakes.

Drosophila oral feeding survival assay

Oral toxin-feeding survival assays were conducted based on the *Drosophila* bacterial intestinal infection protocol by Alameh *et al*. (37). *Drosophila* vials were prepared by placing three 25 mm diameter circles of extra-thick Whatman blotting paper (Bio-Rad Laboratories, catalog #1703965) at the bottom of the vials and capping them with a cellulose plug. Bacterial toxins and toxin components were added in 50 mM sucrose solution at a concentration of 1 μg/mL, which falls within the range of the concentration of plasma-circulating toxin components in infected mammals during the late stages of infections, and as previously tested (37). This concentration is also within the range of toxins found in water used to rinse food contaminated with bacteria (38). Whatman paper was not changed during the experiment.

Depending on the experimental condition, flies were exposed to 50 mM sucrose solution alone or sucrose solution containing 1 μg/mL of bacterial toxins. Flies were anesthetized via CO_2, separated by sex, and placed in vials containing 2.5 mL sucrose solutions. Each vial contained at least 20 males per condition. All experiments were performed with male flies to avoid a potential variability in survival and immunity due to confounding sex differences. Because the immunity of flies changes with age (24, 39-41), the tested flies were of mixed ages. Experimental vials were incubated at 30° C and checked at least 2 times per day to monitor the time of death. Insects that ingest toxins are also exposed to microbes. Other previous studies tested the immunity of *D. melanogaster* infected with Gram-positive and -negative bacteria as well as fungi at 30° C. Thus, we designed our experimental intoxication model consistent with such studies (42-45). Although the optimal temperature for the flies ranges from 22 to 25° C, the toxin-treated flies were maintained at 30° C. The incubation temperature of 30° C after infection was also chosen because the optimal growth temperature for the pathogenic bacteria whose toxins, we used in this study is 30° C or higher.

Data analysis

We provided two types of quantitative analyses of our data: parametric and non-parametric. Parametric analysis was calculated as a *P* value, accounting for differences between the entire two Kaplan-Meier curves. We also provide non-parametric analysis, which does not make any assumptions, and the central tendency is measured as the differences in median

Table 1. Toxins used in this study.

Toxin	Toxin type	Pathogen (Gram + or -)
Alpha (α) toxin	Pore-forming	*Clostridium septicum* (+)
Epsilon (ε) toxin	Pore-forming	*Clostridium perfringens* (+)
Adenylate cyclase	Converting ATP to cAMP	*Bordetella pertussis* (-)
Pertussis toxin	ADP-ribosylating	*Bordetella pertussis* (-)
Cholera toxin	ADP-ribosylating	*Vibrio cholerae* (-)
Diphtheria toxin	ADP-ribosylating	*Corynebacterium diphtheriae* (+)
Toxin A	Glycosylating	*Clostridium difficile* (+)
Toxin B	Glycosylating	
Pasteurella multocida toxin	Deaminating	*Pasteurella multocida* (-)
Lipopolysaccharide (LPS)	Outer membrane endotoxin	*Escherichia coli* (-)
LPS	Outer membrane endotoxin	*Salmonella typhimurium* (-)
LPS	Outer membrane endotoxin	*Salmonella minnesota* (-)
Lipid A	Component of LPS	

survivals between two curves. Data analysis was conducted using GraphPad Prism software. All *P*-values reported are products of the respective positive control to a single experimental condition using two statistical analyses: the Log-rank (Mantel-Cox) and the Gehan-Breslow-Wilcoxon tests. An alpha of 0.05 was deemed the threshold for significance. We report *P* values adjusted by the Bonferroni correction. A change in median survival was reported. Since the chance of dying in a small-time interval was different early in the study and late in the study, the values for the 95% CI of the ratio of median survivals were not meaningful and were not reported. Each insect experiment shown is representative of three independent experiments with 20 flies per condition. Figure 2N A shows the average and standard errors of median survival of flies exposed to each toxin. Predictions of delivery,

Results

Determination of the effect of toxins from human pathogens on the survival of *Drosophila*

Insects can be exposed to various toxin-producing human bacterial pathogens. The effect of ingested toxins on insects has not been extensively studied. Thus, we tested the response of *D. melanogaster* to twelve orally fed exotoxins and endotoxins produced by ten human bacterial pathogens. These toxins were chosen because they originate from various Gram-negative and Gram-positive bacteria with diversity in molecular structures and biochemical functions. Toxins and their characteristics are summarized in Table 1.

Wild-type (WT) fly survival varied when exposed to the various toxins of human bacterial pathogens (Fig. 1). Pore-forming α- and ε-toxins from *C. septicum* and C. *perfringens* significantly increased the median survival of flies by 85.5 h and 84.5 h, respectively (Fig. 1A-B). Conversely, B. pertussis adenylate cyclase toxin and *E. coli* LPS decreased the median survival by 94.5 h and 66 h, respectively (Fig. 1C-D). All other tested toxins did not significantly affect the survival of WT flies (Fig. 1E-M). Among those is *Salmonella* LPS, consisting of lipid A and a polysaccharide, as well as lipid A alone. These results show that only some toxins adversely affect *Drosophila* survival, and that insects are innately insensitive to many microbial toxins.

Bacterial toxins affect *Drosophila* survival through stress response regulator Jra

To determine if Jra is necessary for the response of *Drosophila* to microbial toxins, we measured the survival of a Jra mutant strain of *D. melanogaster* relative to that of WT flies following exposure to the same toxins as in Fig. 1. Jra is a stress-responsive transcription factor consisting of a bZIP DNA binding domain and a transcription activation domain. The mutant strain expresses a dominant-negative Jra allele consisting of its bZIP domain absent of the transcription activation domain (46-49).

The extended survival of WT flies in response to pore-forming toxins was negated in Jra mutant flies. After exposure to α- and ε-toxins, the extension of survival decreased from 85.5 h and 84.5 h, respectively, in WT flies (Fig. 1A-B) to 24 h and 36 h, respectively, in Jra flies (Fig. 2A-B). Of the toxins that shortened the survival of WT flies, *B. pertussis* adenylate cyclase and *E. coli* LPS further decreased the survival of Jra flies by 117.5 h and 93 h, respectively (Fig. 2C-D). The toxins that did not affect the survival of WT flies all shortened the survival of Jra flies by a range of 24h to 108.5 h, except the Diphtheria toxin, which did not affect either strain (Fig. 2E-M). Notably, *Salmonella* LPS and lipid A (Fig. 2E, F, M) shortened the survival of Jra flies by 86.5h to 98.5 h, in contrast to having no effect on WT flies (Fig. 1E, F, M), indicating that the toxicity of LPS in Jra flies is likely caused by the lipid A component. Collectively, these results suggest that Jra is necessary for the response of *Drosophila* to many toxins of various human pathogens (Fig. 2N).

Validation of Jra's role in response to ε-toxin

In addition to expressing a partial Jra gene sequence that codes for a dominant negative form of a protein, the Jra 7218 strain also harbors loss of function point mutations in genes y and

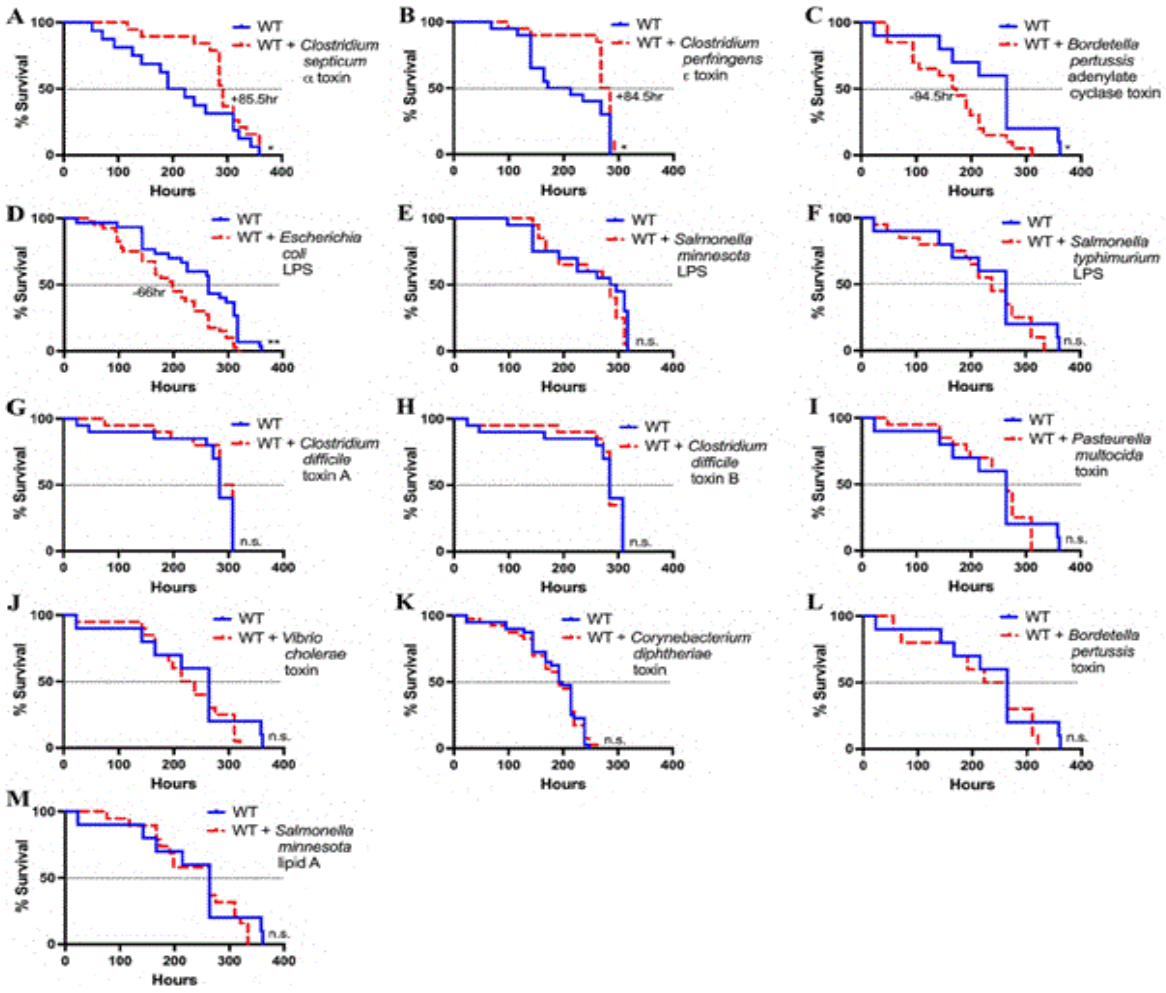


Figure 1: Survival of *D. melanogaster* in response to toxin exposure. The survival of male Oregon-R wild-type (WT) flies was measured in the absence and presence of orally administered bacterial toxins: *Clostridium septicum* α-toxin (A), *Clostridium perfringens* α-toxin (B), *Bordetella pertussis* adenylate cyclase toxin (C), lipopolysaccharide (LPS) from *Escherichia coli*, *Salmonella typhimurium* and *minnesota* (D-F), *Clostridium difficile* toxins A and B (G-H), *Pasteurella multocida* toxin (I), *Vibrio cholerae* toxin (J), *Corynebacterium* diphtheriae toxins (K), *Bordetella pertussis* toxin (L), and *Salmonella minnesota* Lipid A (M). All toxins were tested orally at 1 µg/ml in a sucrose solution. The effect of each toxin on fly survival was compared to toxin-free control treatments. Positive and negative numbers indicate changes in the median survival, shown as a dotted horizontal line. Asterisks indicate statistically significant differences between treatment and control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; n.s., not significant.

w, called y^1 w^{1118}, respectively (46-49). We investigated whether the loss of response to ε-toxin in Jra strain 7218 is due to y^1 mutation by testing the survival of another fly strain, 7217, which expresses the same dominant negative form of Jra in the genetic background w^{1118} and wild-type y gene (46-49). We observed that similar to the Jra 7218 strain, the survival of the Jra 7217 strain was unaffected by α-toxin (Fig. 3A and C). This result shows that mutation in the y gene does not affect the response to α-toxin.

To further investigate whether the loss of response to α-toxin in Jra strain 7218 is due to w^{1118} and y^1 mutations, we tested the sensitivity of another Jra mutant strain, 3273, harboring wild-type y and w genes in a different genetic background cn^1 bw^1 $speck^1$/CyO Jra^{IA109} (50). This strain carries Jra with a loss of function point mutation, where a single base change at position 651 of the Jra sequence introduces a stop codon. Therefore, the mutation results in a truncated Jra protein lacking the DNA-binding and dimerization domain (51). We observed that, similar to Jra strain 7218, the survival of Jra strain 3273 is unaffected by α-toxin (Fig. 3B-C). This further supports the finding that Jra is responsible for toxin response in flies.

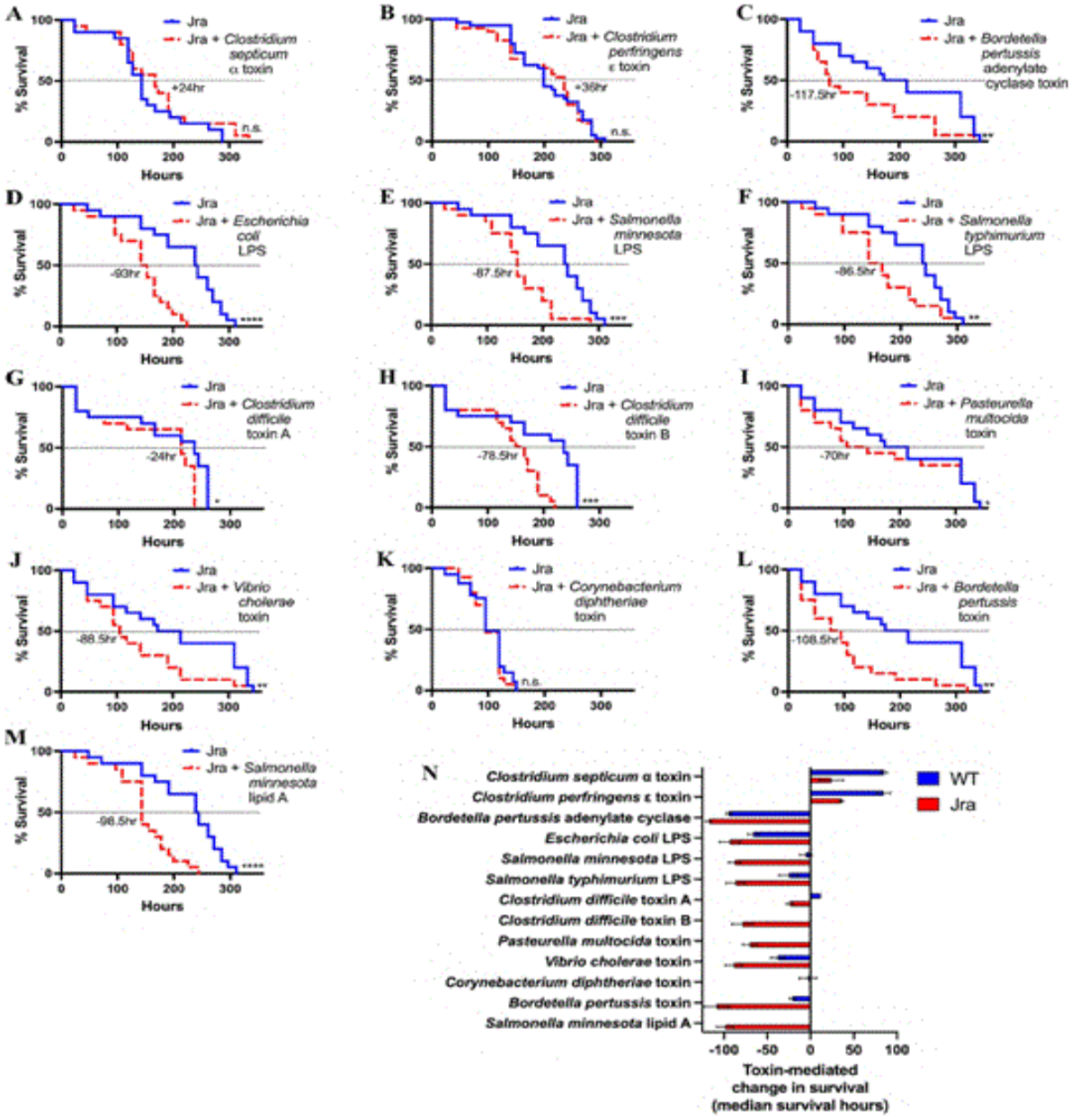
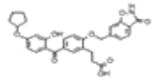
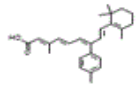
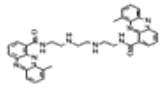
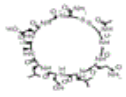
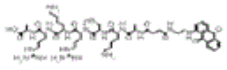


Figure 2: Effect of Jra mutation on the survival of *D. melanogaster* in response to toxin exposure. (A-M) The survival of male Jra mutant flies was measured in the absence and presence of orally administered bacterial toxins, as in Figure 1A-M. Asterisks indicate statistically significant differences between treatment and control, as in Figure 1. (N) Summary of toxin-mediated changes in the survival of WT (Fig. 1A-M) vs. Jra mutant flies (Fig. 2A-M): positive and negative values represent longer and shorter survival, respectively, relative to untreated control. *P* as in Figure 1.

Table 2. Predicted physicochemical and biopharmaceutical properties of putative Jra inhibitors.

Compound structure	Compound identifier	Jun %Inhibitory [conc] or Kd	Diffusion coefficient (cm^2/s x 10^5)	log P	Log (air-water partition coefficient) atm*m^3/mol	Permeability through human skin (cm/s x 10^7)	Intrinsic water solubility (mg/mL)	Volume of distribution (L/kg)	Cellular permeability classification	Absorption Risk (0-8 range). Exceeds 4 undersirable.
	T-5224	10 µM, 50%	0.569	4.33	-12.176	54.449	0.002	0.429	High (62%)	1.953
	SR 11302	1 µM, >81%	0.594	7.55	-8.453	287.227	0.0000206	0.298	High (99%)	2
	MLN 944 (XR 5944)	25 µM, 100%	0.506	4.5	-15.79	24.959	0.084	5.807	Low (61%)	4.085
	Ac-c[Cys-Gly-Gln-Leu-Asp-Leu-Ala-Asp-Gly-Cys]-NH2	8 µM, 50%	0.366	-2.4	-17.008	28.075	2.356	0.275	Low	6.5
	Anthraquinone-DBD	16.8 µM, 74%	0.33	-1.6	-17.336	61.748	27.09	0.614	Low	6.5

Discussions

Our study demonstrates that insects tolerate toxins of many human microbial pathogens, potentially resulting in their greater opportunity to transmit the disease-causing microbes. Our study begins to uncover the mechanism of toxin response in insects and shows the necessity of Jra in toxin response.

Numerous human pathogens exert their harmful effects by expressing toxins. Unlike known effects of insecticidal microbial toxins, such as the pore-forming Cry toxin of *Bacillus* thuringiensis (52), very little is known about the effects of toxins of human microbial pathogens on insects. Since host targets of toxins and immune responses are evolutionarily conserved between mammals and insects, *D. melanogaster* has been utilized as a model organism to study the function of toxins of human pathogens and host sensitivity (53-64). In most of these studies, bacterial toxins were genetically expressed in flies and adversely affected the developmental and immunological processes in *D. melanogaster*. For example, just like in humans, it was shown that i) tetanus toxin and *Botulinum* neurotoxins disable *Drosophila* neuronal function by targeting fly SNARE homolog (53-55), ii) Diphtheria toxin and *Pseudomonas* exotoxin A inhibit protein synthesis in *D. melanogaster* by catalyzing the ADP-ribosylation of insect homolog of eEF-2 (54, 56), and iii) cholera and Pertussis toxins catalyze the ADP-ribosylation of a fly homolog of Gs protein, which activates cellular adenylate cyclase and results in a toxic level of cyclic adenosine monophosphate (cAMP) (57-59). Other secreted toxins studied by genetic expression in flies include AvrA of *S. typhimurium* (60) and CagA of *Helicobacter pylori* (61). In other studies, the contribution of toxins to bacterial pathogenicity was demonstrated by infecting flies with toxin-expressing and toxin-deficient bacterial strains. This was done for cholera toxin (62) and pore-forming hemolysins from S. *aureus* and *Serratia marcescens* (29, 63, 64).

However, only a few studies tested the effect of toxins on flies by a physiologically relevant feeding assay. This was accomplished by exposing flies to purified recombinant cholera and anthrax toxins (37, 65). In contrast to toxins' effect on mammals, orally ingested anthrax and cholera toxins did not affect the survival of flies, and the anthrax toxin component activated *Drosophila* resistance to bacteria through immune pathway mechanisms. Our study tested the effect of twelve toxins of human bacterial pathogens on the survival of flies by the feeding assay and investigated the mechanism of toxin response in flies. When ingested, most of these toxins do not affect the survival of flies at 1 µg/mL. Notably,

the enhanced survival of *Drosophila* fed with pore-forming toxins (Fig. 1A-B) resembles the hormetic response, a phenomenon by which adaptive responses of organisms or cells to low dosages of stresses enhance their survival and stress resistance (66).

Several confounding factors limit the interpretations of our study. Only one concertation, 1 μg/mL, was tested for each toxin, and future tests should determine possible lethal and sub-lethal doses for each toxin. In addition, although each experiment was performed at least three times, they were performed with flies of various ages. This was done because the immunity of flies changes with age (24, 39-41). Within an experiment, the age distribution of flies in control and experimental conditions is similar. However, the age distribution may not be the same the next time the experiment is performed. Thus, overall survival may vary from experiment to experiment. Notwithstanding, the effect of microbial toxins on survival was reproducible, as seen by error bars for median survival in Figs 2N and 3C. Additionally, changing Whatman papers during the experiment was not practical; thus, one of the factors that limited fly survival in our experiments was the evaporation of the sucrose solution. Moreover, sucrose solution is not a well-balanced food, and flies' survival is not expected to be as long as on the nutrient-rich fly food. Another factor that could affect flies' survival in our experimental design is the deposition of feces and eggs on the surface of the Whatman paper, which could potentially limit access to food. Other confounding limitations are the possibility that the Jra mutation may affect the amount of food and microbial toxins consumed by flies.

In humans, Jun has been shown to have several functions. A robust Jun expression has been observed in all cell lines from patients with classical Hodgkin lymphoma and anaplastic large-cell lymphoma (67). Additionally, Jun is essential for neuronal microtubule assembly and apoptosis (68), activation of Jun-mediated transcription cell cycle regulation (69), protective immunity through increased CD47 and PD-L1, and involvement in toxins signaling (60, 70, 71).

Our study supports the role of Jra in response to many microbial toxins. *Drosophila* Jra expression was induced in response to cholera toxin and LPS treatments (33, 34). Moreover, the JNK (Jun N-terminal Kinase) pathway, a MAPK cascade known to activate Jra, is induced by *H. pylori* CagA toxin (72). We propose that insects respond to toxins through Jra activity, which allows mounting the innate immunity to toxins and toxin-producing pathogens. To evade this Jra-mediated toxin immunity, other toxins may block and inactivate Jra-response: the *Drosophila* JNK pathway is inhibited by anthrax toxin Lethal Factor and by *Salmonella enterica* toxin AvrA, similarly to how they act in mammals (60, 70, 71). In human cells, anthrax toxin Lethal Factor acts as a proteinase that decreases Jun levels. Such levels were restored with a 26S proteasome inhibitor, indicating that anthrax toxin promoted the degradation of Jun protein through a proteosome-dependent pathway (70, 71). It was also shown that the secreted *S. typhimurium* effector protein AvrA possesses acetyltransferase activity toward specific MAPKKs and potently inhibits JNK-Jun and NF-κB signaling pathways in *Drosophila* and mice (60).

Previous studies demonstrated that Jra could promote longevity in *Drosophila* (73). Various stresses, such as pathogens and heat, activate Jra through JNK (bsk) protein (74). As a result, Jra regulates and promotes many physiological processes that influence insect homeostasis, including cytoprotection, wound healing, cell proliferation, and the extension of the lifespan (73, 75). Previous studies have shown that Jun activates the expression of a set of genes that mediate the synthesis of fatty acids and lipids, thus driving lipogenesis (3, 76). Other studies showed that the pore-forming α-toxin of *S. aureus* and aerolysin of *Aeromonas hydrophila* induce lipogenesis and restoration of the bilayer integrity (77, 78). We hypothesize that α-toxin, at the dosage examined in our study, affected the survival of flies by activating Jra, which induced lipogenesis and epithelial septate junction repair, allowing for cytoprotection and extension of survival. Meanwhile, studies revealed that prolonged longevity (hormetic response) involves multiple integrative signal transduction processes that are dependent on types of stressors and dosage levels (79, 80). Hence, elucidating the comprehensive mechanism of prolonged survival of flies warrants future in-depth investigation with varying doses of toxins.

This study identifies the insect Jra as a new protein target for future insecticidal compounds capable of protecting mammals from pathogens-vectoring insect pests. A literature survey was performed to facilitate future optimization of promising compounds displaying Jra antagonism, resulting in several hits against the mammalian homolog, Jun. A short list exemplifying starting points for chemical space for optimization of Jra antagonism is proposed in Table 2 based on published selectivity and potency (81), coupled with *in silico* evaluation of permeability potential, biological distribution characteristics, and chemical/metabolic stability (i.e., ADME properties, using ADMET Predictor v. 10.3) as additional critical governing factors (82, 83). Additionally, since Jra is known to form complexes with other transcription factors, it is pertinent to consider compounds within the context of equilibrium interactions of these transcription response elements. To bind effectively to the target, an essential putative Jra antagonist must be selective and overcome potential off-target competitive interactions. From a stoichiometric perspective, small molecule antagonists possess physical, chemical, and biopharmaceutical properties that enable formulation and delivery at pharmacological quantities, which can outcompete known probabilities, such as hetero-dimerization and interactions with off-target components. Within this context, most prior work has focused on disrupting the interaction of Jun with DNA or with Jun-binding heterodimers. These functional regions on the surface of Jun represent defined target surfaces suited for small molecule inhibitors. While published drug candidates targeting mammalian Jun have historically been small molecules, earlier work on short peptide-based disruptors may offer additional options.

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Declarations

a. Conflict of interest: NA

b. Ethical approval: NA

c. Clinical trial registration: NA

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