A conserved role for a GATA transcription factor in regulating epithelial innate immune responses

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Innate immunity is an ancient and conserved defense mechanism. Although host responses toward various pathogens have been delineated, how these responses are orchestrated in a whole animal is less understood. Through an unbiased genome-wide study performed in *Caenorhabditis elegans*, we identified a conserved function for endodermal GATA transcription factors in regulating local epithelial innate immune responses. Gene expression and functional RNAi-based analyses identified the tissue-specific GATA transcription factor ELT-2 as a major regulator of an early intestinal protective response to infection with the human bacterial pathogen *Pseudomonas aeruginosa*. In the adult worm, ELT-2 is required specifically for infection responses and survival on pathogen but makes no significant contribution to gene expression associated with intestinal maintenance or to resistance to cadmium, heat, and oxidative stress. We further demonstrate that this function is conserved, because the human endodermal transcription factor GATA6 has a protective function in lung epithelial cells exposed to *P. aeruginosa*. These findings expand the repertoire of innate immunity mechanisms and illuminate a yet-unknown function of endodermal GATA proteins.

gene expression | innate immunity | infection | ELT-2 | *Pseudomonas aeruginosa*

The innate immune system is an evolutionarily conserved defense mechanism, in which pathogen-associated molecular patterns (PAMPs) or infection by products, are recognized by receptors leading to regulated expression of immune modulators and antimicrobial molecules (1–3). A central mechanism in most studied organisms involves the axis formed by PAMP-recognition Toll-like receptors and NF-κB transcription factors (4, 5). However, *Caenorhabditis elegans* lacks the Toll-NF-κB axis (6) and yet effectively protects itself from diverse pathogens, underscoring the importance of other mechanisms in innate immunity. *C. elegans* was shown to protect itself from localized bacterial infections, which rely on the same virulence factors necessary for infection in vertebrates (7), through the function and integration of several conserved signal transduction pathways. These include the Sma/TGF-β, insulin, and p38 mitogen-activated protein kinase pathways (8–11). However, to date, no transcription factor has been directly shown to mediate the function of any of these pathways in regulating transcriptional responses to infection.

GATA zinc-finger transcription factors play critical roles in development and differentiation in both vertebrates and invertebrates. Six family members exist in vertebrates, of which GATA1-3 are crucial for hematopoiesis (12), and GATA4-6, for mesoendodermal development (13). An additional role was identified in *Drosophila* larvae, where an early meso- and endodermal development GATA factor, Serpent (14), regulates a systemic induction of antimicrobial peptides in response to systemic infection (15). Similar to vertebrates and *Drosophila*, *C. elegans* GATA proteins are essential for endodermal development (12). Acting sequentially in the E cell lineage as a regulatory cascade, GATA transcription factors direct the formation of the worm intestine (16). The last in the cascade are *elt-2* and *-7*, which are restricted in their expression to the intestine and are responsible for terminal differentiation of intestinal cells. Of this pair, *elt-2* continues to be expressed during adulthood (17). Although GATA transcription factors are expressed during adulthood or in mature tissues, in other animals as well, very little is known about their postdevelopmental roles.

Here we show that in both *C. elegans* and human cells, an endodermal GATA transcription factor has a postdevelopmental role in local defense against the human Gram-negative bacterial pathogen *Pseudomonas aeruginosa*. Using whole-genome gene expression and functional analyses in *C. elegans*, we identified the GATA transcription factor ELT-2 as a major regulator of an early protective response to *P. aeruginosa*. We found that the human homolog, GATA6, protects lung epithelial cells from a similar infection, describing a conserved role for GATA transcription factors in mediating local epithelial responses to infection.

Results

Conserved and *C. elegans*-Specific Gene Expression Responses to *P. aeruginosa* Infection. To better understand host responses to infection in the context of a whole organism, we characterized gene expression responses of young adult wild-type *C. elegans* to infection with PA14, a clinical isolate of *P. aeruginosa* (18). Using a GFP-expressing PA14 derivative (PA14-GFP), we followed bacterial accumulation in the worm intestine (Fig. 1*A*). Time points chosen for gene expression analyses were 4, 12, and 24 h of exposure, in which bacterial colonization progressed gradually from a primarily pharyngeal localization (Fig. 1*A Top* and data not shown) to densely filling the intestinal lumen, concomitant with distension of the intestinal lumen (Fig. 1*A Bottom*). These time points precede the first deaths (18). At all times, infection was restricted to the alimentary tract. We compared mRNA abundance for >90% of *C. elegans* genes, using spotted PCR microarrays, between animals exposed either to PA14 or to the nonpathogenic *Escherichia coli* strain OP50.

We identified 232 genes whose expression changed in response to infection, using a procedure based on two-way ANOVA. Of these, 197 were induced and 35 repressed (Fig. 1*B* and Table 2, which is published as supporting information on the PNAS web site). Quantitative RT-PCR (qRT-PCR) measurements confirmed microarray results for 49 of 52 responding genes tested (Table 3, which is published as supporting information on the PNAS web site). We examined the tissue distribution of the responding genes using lists of muscle-, germline-, and intestine- enriched transcripts (19–22). Fifty-seven percent of these were gut-enriched, and 8%

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Abbreviations: DUF, domain of uncharacterized function; Esp, enhanced susceptibility to pathogens; qRT-PCR, quantitative RT-PCR; CUB, complement subcomponents C1s/C1r, Uegf, Bmp1.

Data deposition: The microarray data have been deposited in the Stanford Microarray Database (http://smd.stanford.edu) and the Gene Expression Omnibus database (accession no. GSE5584).

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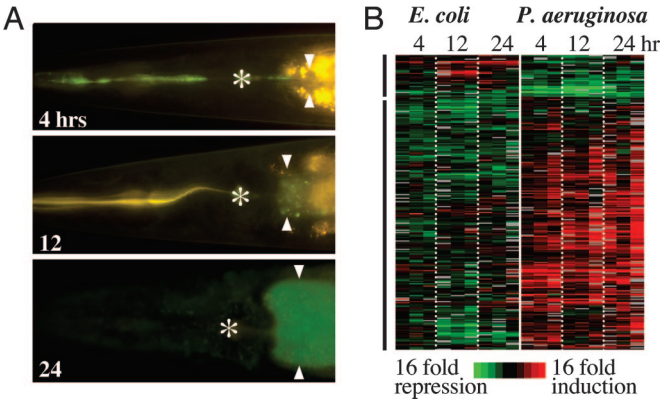

Fig. 1. *P. aeruginosa* accumulation in the worm gut is accompanied by robust gene expression changes in the host. (*A*) Representative images (×400) of worms (10–20 per group) exposed to PA14-GFP for 4, 12, and 24 h. Asterisks mark the posterior pharyngeal bulb; wedges mark the intestinal lumen boundaries. Yellow signal is autofluorescence of intestinal granules; in addition, a yellow pharyngeal signal of an unknown source appeared consistently at the 12-h time point. (*B*) Genes differentially expressed during *P. aeruginosa* infection. Hierarchically clustered expression profiles (rows) for 248 PCR products, corresponding to 232 genes, which are differentially expressed under exposure to PA14 compared with OP50. Data from three independent experiments (columns) are shown for each time point, separated by dotted lines. Vertical bars mark clusters of genes repressed (top) or induced (bottom).

were genes enriched in body muscle, a tissue with a relative mass comparable to that of the intestine. The fraction of transcripts enriched in other tissues was negligible. The preferential response of intestinal genes to infection suggests that the local infection caused by *P. aeruginosa* elicits a response that is mostly local.

The major functional domains in the observed response are summarized in Table 1. Induced genes showed a significant enrichment for members of several conserved gene families: lysozymes, lectins, histones, and genes encoding proteins with a GST domain. A similar enrichment was found for DUF274 genes, representing a family unique to *Caenorhabditis* (DUF stands for domain of unknown function) and for members of a gene family that encode proteins with a CUB-like domain (formerly known as DUF141), a variant of a domain found in diverse proteins, including the C1s and C1r complement system peptidases (CUB stands for Complement subcomponents C1r/C1s, Uegf, Bmp1; see ref. 23). Induction of lysozyme, lectin, and CUB-containing genes describes an expansion of a previous report studying responses to another Gram-negative bacteria (9). The induction of histone genes was preferentially of histone H2A and H2B, suggesting functions other than DNA packaging, which requires equal ratios of all four core histone subunits. It is possible that, in the context of infection, induction of these specific isoforms serves to provide antimicrobial peptide precursors, as reported for various organisms (24, 25). Among the repressed genes, one group, annotated with the Gene Ontology annotation of "lipid metabolism," was enriched (Tables 1 and 2).

We individually assessed the functional significance of 190 of the 232 responding genes by knocking down their expression in adult animals and measuring their sensitivity to PA14, as well as their lifespan on OP50 (*Materials and Methods* and *Supporting Text*, which is published as supporting information on the PNAS web site). This approach allowed us to determine gene contribution to immunity independently of possible functions important for development or normal lifespan. Knocking down the expression of 21 genes resulted in enhanced susceptibility to the pathogen (Esp), yet did not affect lifespan, suggesting they are immunity genes (or esp genes; Table 4, which is published as supporting information on the PNAS web site). By comparison, a similar screen that targeted the majority (2,251) of chromosome I genes by RNAi from the egg stage yielded only four esp genes (B.J.H. and M.-W.T., unpublished results), thus indicating that esp genes are highly enriched among those genes whose expression is modulated by infection. The esp genes included members of multigene families, such as the CUB-containing genes F08G5.6 and F20G2.5. They also included the histone genes *his-10* and *-16*, thus supporting the notion that histone gene induction may provide an immune-specific function, presumably as precursors for antimicrobial peptides. Interestingly, some esp genes were repressed during infection, including a putative fatty acid synthase gene, and *thn-1*, which encodes a homolog of the thaumatin family of plant antifungal proteins. Overall, these functional analyses demonstrate that the identified transcriptional response represents a functional immune response.

Infection Responses Are Distinct from General Stress Responses. It is possible that the gene expression changes we observed during infection were an indirect effect of associated cellular damage and thus represented a general stress response. To examine how similar the infection response is to general stress responses, we compared the list of infection-response genes to ≈1,000 genes previously found to be affected by cadmium, a wide-range toxic metal (26). We found an overlap of 82 genes, including GST, DUF274, and CUB-containing genes (Table 2). However, the greater part of the infection response was distinct from that caused by cadmium and further differed in its functional composition. Notably, only one heat-shock protein was induced by infection, compared to 14 strongly induced by cadmium, whereas histone gene induction, a prominent feature of the infection response, was altogether missing in cadmium response. Within the grossly overlapping portion of

Table 1. Major functional groups represented in the infection response

Functional groups†	No. in genome	No. in analyzed dataset (of 7,308 genes)	No. in infection response list (of 197 induced, or 35 repressed genes)‡	*P* value for enrichment§
Lysozymes	10	9	2	0.02
Lectins	285	64	11	8×10^{-7}
CUB-like	52	29	17	$<10^{-8}$
DUF274	19	10	9	$<10^{-8}$
Histones	76	48	7	2×10^{-4}
GST	70	38	11	$<10^{-8}$
Lipid metabolism	214	119	3	0.016

†Shown are only the major functional groups. Genes lacking a shared and/or clear functional affiliation are not considered here. All members of a group were retrieved from WormBase by using either gene names (i.e., his-*, lys-*) or protein motif match. Lectins include proteins with the C-type lectin, selectin, or galectin domains.
‡Number of genes induced (for the top six categories) or repressed (for the lipid metabolism category); for specific genes, see Table 2.
§*P* values for enrichment were calculated based on the hypergeometric distribution.

infection and cadmium responses, subtler differences were also apparent: among the seven overlapping CUB-containing genes, one (F55G11.2) was induced during infection, but repressed under cadmium exposure, and two (F08G5.6 and F55G11.5) responded much more strongly to infection than to cadmium. This comparison demonstrates that, despite some common features, the infection response is largely distinct from a general stress response.

A prominent stress mechanism in *C. elegans* is the forkhead transcription factor DAF-16, which is necessary for both lifespan extension (27) and increased resistance to infection (11) in *daf-2* insulin pathway mutants. Therefore, we wondered whether DAF-16 could be the regulator of the infection response. We compared the infection-response gene list to a previously reported list of DAF-16 targets (28) and found that, although an overlap existed between the two lists (41 genes), the trends were opposite. Lipid metabolism and thaumatin genes, which are induced by DAF-16, were repressed during infection. Similarly, infection-induced genes, such as lectin, DUF274, and CUB-containing genes, are repressed by DAF-16. DUF-16 function requires its nuclear translocation (27). Using a strain that expresses a functional DAF-16::GFP protein fusion, we observed that, unlike oxidative, starvation or heat stresses, nuclear localization could not be detected in PA14-infected animals (data not shown). Together, these results imply that DAF-16 is not an active regulator of the observed infection response, and that another regulatory mechanism is involved.

A GATA Motif Is Enriched in Promoters of Infection-Response Genes. To identify mechanisms that directly regulate the observed gene expression changes, we searched the proximal promoter regions (500 bp) of infection-response genes for shared DNA motifs, which might mediate binding of transcriptional regulators (*Supporting Text*). We exclusively identified the GATA motif (TGATAAGA; Table 5, which is published as supporting information on the PNAS web site) in 35% of all induced genes and 25% of all repressed genes, compared to a 10% prevalence in proximal promoter regions of 4,000 random genes ($P < 10^{-8}$, hypergeometric distribution). GATA motifs were preferentially localized within the 250-bp more proximal region.

The enrichment for GATA motifs suggested a functional role for this motif in regulating infection responses. Consistent with this, two genes encoding GATA-binding transcription factors were induced during infection: *elt-2* and C18G1.2. This induction was observed in microarray results (Fig. 2*A*) and further verified by qRT-PCR (Table 3). We hypothesized that one or both of these transcription factors could be the GATA-binding protein that modulated the expression of infection-responsive genes.

ELT-2 Functions Postdevelopmentally to Protect Worms from Infection. ELT-2 is essential for normal intestinal development in embryonic and larval stages (17). To test the hypothesis that it has a postdevelopmental role in innate immunity, we determined the effect of *elt-2* knockdown in adulthood on the susceptibility of worms to infection. After exposure to RNAi, *elt-2* expression remained suppressed for >6 days (Fig. 6, which is published as supporting information on the PNAS web site). Reducing *elt-2* expression to 10% of its levels in control-treated animals, as assessed by qRT-PCR, resulted in a significant decrease in survival on PA14 ($P < 0.0001$, log-rank test; Fig. 2*B*). This represented an increased sensitivity to infection and not a general shortening of lifespan (Fig. 2*C*). Comparable results were found in diverse genetic backgrounds, in both sterile and fertile wild-type animals (results not shown), demonstrating that *elt-2* is specifically required for antibacterial defense in the worm. Conversely, we could not detect any significant effect for C18G1.2 knockdown or disruption (results not shown).

The increased sensitivity of *elt-2*(*RNAi*) worms to infection was accompanied by faster accumulation of the pathogen in the worm intestine, as seen in animals grown on PA14-GFP. Fourteen hours

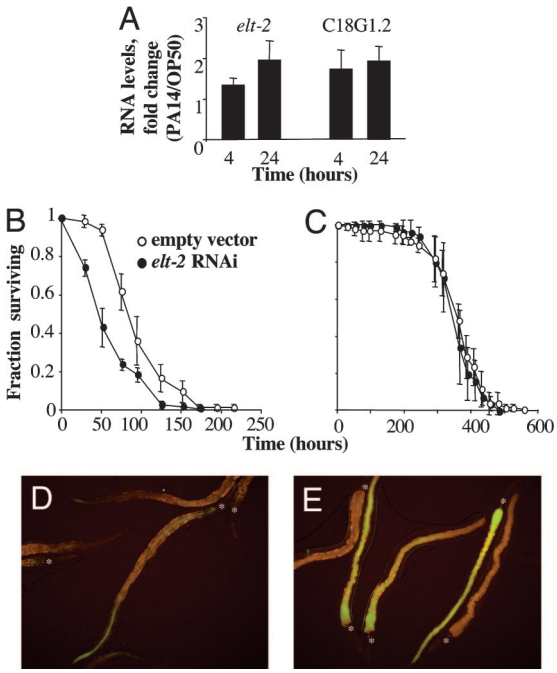

Fig. 2. Functional consequences of *elt-2* expression knockdown. (*A*) Gene expression changes for *elt-2* and C18G1.2 during infection. Means ± SEM for two to three microarray measurements are shown. (*B*) Survival assays for *spe-26* mutants fed with *E. coli* either expressing *elt-2* RNAi (open circles; $n = 102$) or containing the control empty RNAi vector (filled circles; $n = 99$) and subsequently transferred (at time 0) to PA14 lawns. Shown for each curve are means ± SD of the fraction of live animals on each of three plates. (*C*) Lifespan assays for the same experimental groups as above [$n = 85$ for *elt-2(RNAi)* animals; $n = 92$ for controls]. Worms were exposed, after RNAi treatment, to kanamycin-killed OP50-1. (*D* and *E*) Faster intestinal accumulation of *P. aeruginosa*. Representative images (×200) of *glp-4;rrf-3* mutants grown on control (*D*) or *elt-2* -RNAi-expressing bacteria (*E*). Asterisks mark the pharynx.

after initial exposure, 2.6% and 5.9% of control animals in two independent experiments showed an intestine completely filled with bacteria (Fig. 2*D*). In contrast, 19% and 57% of *elt-2*(*RNAi*) animals, respectively, showed filled intestines (Fig. 2*E*; $P = 0.02$, χ^2 test, 40 and 100 animals per group, respectively). This suggests that ELT-2 functions to slow down bacterial accumulation in the worm intestine.

***elt-2* Knockdown Impairs Infection-Specific Gene Induction.** To further investigate the role of *elt-2* during infection, we asked whether the susceptibility of *elt-2*(*RNAi*) worms was correlated with changes in expression of infection-response genes. We used qRT-PCR to determine the effect of *elt-2* knockdown on the expression of three infection-induced genes that have GATA motifs in their promoters: *lys-2*, a member of the lysozyme family of bacterial-envelope hydrolyzing enzymes, and two CUB-containing genes, F55G11.2 and F08G5.6. Of these, F08G5.6 by itself showed a substantial contribution to infection resistance (Table 4). Knocking down *elt-2* expression in adult animals significantly reduced basal expression of the three genes (Fig. 3*A*). It additionally abolished their induction after a 24-h exposure to PA14 (for F08G5.6 and F55G11.2) or even changed that induction to repression (for *lys-2*; Fig. 3*C*). These results suggest that the three genes are targets of ELT-2 and provides a link between ELT-2-dependent gene expression and the functional consequences of its inactivation.

In contrast to the three infection-response genes, the expression of five general intestinal genes, including GATA-regulated genes (*ges-1*, *ifb-2*; ref. 17) and genes that contribute to intestine structure development and maintenance (*let-413*, *eps-8*, and *gob-1*; refs.

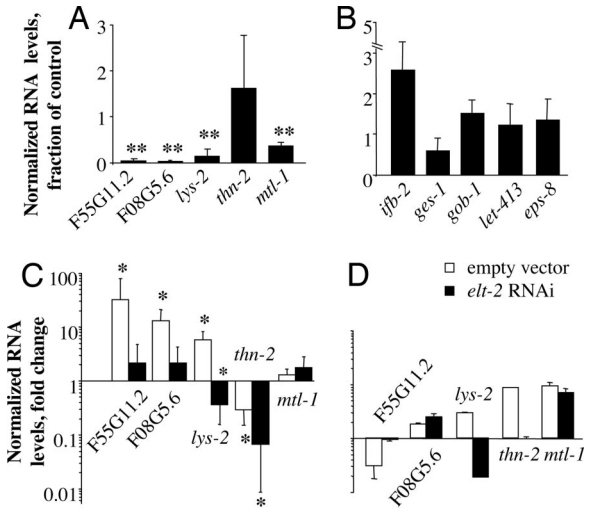

Fig. 3. *elt-2* knockdown selectively affects the expression of infection-response genes. RNA levels were measured by using qRT-PCR. Excluding *D*, columns represent means ± SD for three separate experiments. (*A* and *B*) Effects on constitutive expression. Shown are normalized fractions of specific RNA levels in *elt-2(RNAi)* animals relative to control-treated animals. Double asterisks mark significant decreases ($P < 0.001$; *t* test). (*A*) Infection-response genes. (*B*) Intestine functional and structural markers. (*C* and *D*) Effects on induced expression. (*C*) Infection responses. Normalized fold changes in animals exposed for 24 h to PA14 compared with those exposed to OP50-1. Open columns represent control-treated animals; filled columns represent *elt-2(RNAi)* animals. Asterisks mark significant changes ($P < 0.02$; *t* test). (*D*) Cadmium responses. Normalized fold changes in animals exposed to 100 μM cadmium for 24 h relative to no-cadmium controls. Columns are as described above. Shown are results of one experiment (means ± SEM). The results presented were obtained in *spe-26* worms. Wild-type worms showed similar trends.

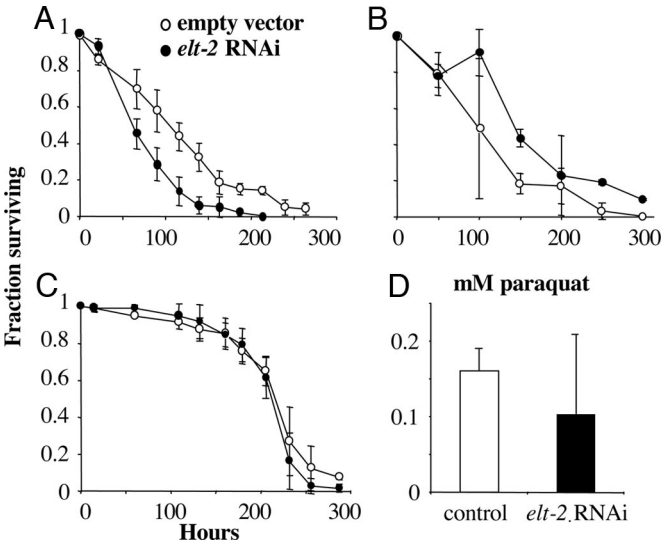

Fig. 4. Consequences of *elt-2* knockdown for protection from infection vs. general stress conditions. Survival assays for *cdc-25.1*-RNAi-sterilized wild-type worms, fed with *elt-2*-RNAi-expressing bacteria (filled) or control bacteria (open) and subsequently subjected to *P. aeruginosa* infection (*A*), oxidative stress (*B*), cadmium stress (*C*), or heat shock (*D*). (*A*) Killing by *P. aeruginosa*. Means ± SD of fraction of live animals on each of three plates. Control animals ($n = 90$); *elt-2(RNAi)* animals ($n = 110$). (*B*) Sensitivity to oxidative stress. Fraction survival as a function of paraquat concentration (6-h exposure) for control and *elt-2(RNAi)* animals. Shown are means ± SD for experiments performed in duplicate (18–45 animals per point). (*C*) Sensitivity to cadmium. Means ± SD of the fraction of live animals on each of three plates [control, $n = 75$; *elt-2(RNAi)*, $n = 85$] are shown. Cadmium concentration was 100 μM, in which RNAi directed against *cdr-1* resulted in a measurable increase in sensitivity (results not shown). (*D*) Sensitivity to heat stress. Means ± SD of the fraction of live animals on each of three plates subjected to 37°C for 17 h [control, $n = 69$; *elt-2(RNAi)*, $n = 66$] are shown.

29–31), was largely not affected by *elt-2* knockdown (Fig. 3*B*). Furthermore, knocking down the expression of any of these genes in adulthood (we did not test *ges-1*) did not affect susceptibility to PA14 (Fig. 7, which is published as supporting information on the PNAS web site). Together, these results indicate that *elt-2* knockdown in adult animals increases susceptibility to infection not through the impairment of general intestinal function but through its specific role in regulating immunity genes.

elt-2 did not contribute to infection-associated gene repression, as observed for *thn-2*, a thaumatin gene that contains a putative GATA factor-binding site in its promoter and that was repressed during infection. *elt-2* knockdown did not decrease *thn-2* basal expression (Fig. 3*A*) or abolish its repression during infection (Fig. 3*C*); it even exacerbated this repression, suggesting that ELT-2 positively regulates the expression of *thn-2* during infection, and that other factors may be responsible for its repression. Finally, the expression of some ELT-2-dependent genes remained unaltered during infection. *mtl-1*, a GATA-containing metallothionein gene that is regulated by ELT-2 under cadmium exposure in larvae (32), did not respond to infection and was not affected by *elt-2* knockdown (Fig. 3*C*).

ELT-2 Functions in Adult Worms as an Immune-Specific Regulatory Mechanism. To further test the specificity of *elt-2* in inducing infection responses, we analyzed gene expression for the confirmed ELT-2 targets described in Fig. 3*C* after exposure to cadmium (Fig. 3*D*). With the exception of *lys-2*, which was similarly induced by cadmium and infection in an ELT-2-dependent manner, these genes presented qualitatively different responses to cadmium and to infection (Fig. 3 *C* and *D*). Similarly, the contribution of *elt-2* to their expression was different under the two conditions, further demonstrating an infection-specific regulatory function.

Consistent with the results described above, we found that *elt-2* expression is necessary for protecting adult animals from infection but not from general stress. We assayed this in wild-type worms made sterile by *cdc-25.1* silencing, in which we observed the greatest differences between control and *elt-2(RNAi)* animals, examining resistance to a variety of stress conditions causing detrimental effects over a wide range of time. Whereas *elt-2(RNAi)* animals were significantly more sensitive to PA14 infection than controls ($P < 0.0005$; log-rank test), they were not more sensitive to oxidative stress caused by paraquat, 37°C heat stress, or exposure to cadmium (Fig. 4). We therefore propose that in the adult animal, ELT-2 functions mainly in regulating protective antimicrobial responses.

Human GATA6 Is Important for Protecting Lung Epithelial Cells from Infection. The identification of ELT-2 as a local intestinal regulator of immune responses in *C. elegans* prompted us to ask whether endodermal GATA transcription factors perform similar roles in humans. The GATA zinc finger domain of ELT-2 most resembles those of human GATA4–6 (33), which, like ELT-2, are central factors in endoderm development (13, 34). In adults, *GATA4* and *GATA6* are both expressed in endodermally derived tissues, including the small intestine and the lung (35, 36).

To investigate the possible involvement of GATA4 and GATA6 in local infection responses in human cells, we used A549 lung epithelial cells infected with PA14. We found by qRT-PCR that *GATA6* was induced by >4-fold upon infection (Fig. 5*A*). In contrast, *GATA4* expression did not change (data not shown). We used small inhibitory RNA to knock down the expression of *GATA6* in these cells (Fig. 5*A*) and measured their ability to withstand infection. *GATA6* knockdown resulted in significantly

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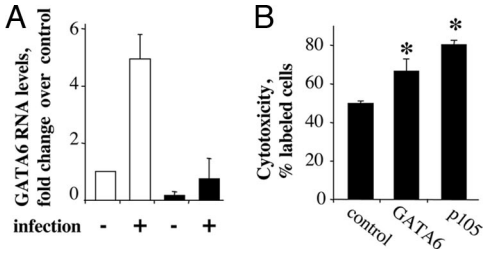

Fig. 5. *GATA6* is important for protecting human epithelial cells from *P. aeruginosa* infection. (*A*) *GATA6* is induced upon infection of A549 cells. Fold changes of GATA6 RNA levels in A549 cells, treated with *GATA6* RNAi (filled bars), or transfection reagent alone (open bars), and after exposure to PA14 or to PBS alone. Fold changes are over control-treated noninfected cells (set to 1). RNA levels were measured by qRT-PCR. Shown are means ± SD of two experiments. (*B*) *GATA6* knockdown increases cells' susceptibility to infection. Percentage of damaged membrane-permeabilized cells, marked by Trypan blue accumulation, of the total number of cells. Counts were performed on randomly captured images (109–187 cells per image), each of a different well in a six-well plate. Shown are means ± SD for three to five wells, in one of two experiments with similar results. Statistically significant values (*t* test; $P < 0.01$) are marked with asterisks.

increased PA14-induced cytotoxicity within 1 h of exposure to diluted log-phase bacteria (Fig. 5*B*). This cytotoxicity was infection-specific, because GATA6 knockdown was not cytotoxic by itself (data not shown). An even larger increase in cytotoxicity was observed in infected cells treated with siRNA directed against the NF-κB subunit gene *p105*, serving as a positive control. Cytotoxicity is probably not attributed to secreted *P. aeruginosa* toxins, which are mostly secreted in stationary phase. The short exposure time sufficient to cause cytotoxicity further excludes substantial accumulation of such toxins in the medium, suggesting that *GATA6* provides protection from the pathogen itself.

As with ELT-2, we expect GATA6 to contribute to protecting cells through gene regulation. Mining of published data of genes responding to infection in lung epithelial cells (37, 38) revealed a high prevalence of the GATA motif (>75%) in strongly induced genes, suggesting that GATA-dependent regulation is common. Together, these results provide evidence that *GATA6* is important for protecting human lung cells during infection.

Discussion

The identification of ELT-2 as a key regulator of a robust infection transcriptional response describes a representative of a regulatory level that was until now missing in the worm, thus connecting protective signal transduction pathways and immune gene induction. Importantly, our experiments in human lung epithelial cells suggest that the main function we identified for ELT-2 in *C. elegans* is evolutionarily conserved. Our findings are consistent with a role for *GATA6* in protective local epithelial responses in the lung and potentially in other endodermal tissues such as the intestine. This is different from previously described functions of GATA proteins in hematopoietic differentiation (39) or the systemic anti-infection responses of *Drosophila*'s *serpent* (15).

P. aeruginosa infection in the worm is localized to the alimentary tract, similar to other human bacterial pathogens that infect *C. elegans* (40). The transcriptional response that we delineated to this infection is apparent within 4 h of exposure to the pathogen, and 57% of the responding genes are primarily expressed in the intestine, suggesting that the major part of the response is associated with pathogen entry and is a primary and local intestinal response.

Among the responding genes are members of gene families known or suspected to take part in various levels of innate immune functions: lectins are known to serve as pathogen recognition proteins in other organisms (41); lysozymes are known for their bacterial hydrolyzing activity; histones, based on the preferred induction of specific isoforms and functional analyses, might serve as precursors for antimicrobial peptides; and two formerly uncharacterized gene families, which encode proteins with the DUF274 or CUB-like domains. It is difficult to demonstrate functional significance for responding genes by single gene knockdown because of redundancy within multigene families. For example, we failed to observe a significant effect on sensitivity to infection when knocking down the expression of either of two tested lysozymes, although one of them can confer resistance when overexpressed (9). However, knockdown of two CUB-containing genes did result in a marked sensitivity to infection, suggesting a greater importance for these genes in protecting worms from infection or, alternatively, less functional redundancy despite their presumed structural similarity. The existence of the CUB domain variant raises the intriguing possibility that this family functions in some way similar to complement-associated proteases, but this remains to be tested.

Overall, a considerable subset of the observed responses is distinct from general stress responses, such as cadmium exposure (26), or from expression patterns associated with general stress mechanisms, such as those attributed to DAF-16 (28). Because DAF-16 is required for *daf-2*'s resistance to bacterial infection (11), its disassociation with the observed infection response suggests that it contributes to this resistance through a parallel route, probably affecting the basal state of infected worms rather than induced responses, either by affecting constitutive expression of antimicrobial genes or, less specifically, by affecting general well being.

RNA level measurements for a small set of ELT-2-dependent infection response genes further highlighted the differences between responses to infection and to cadmium and the distinct contribution of *elt-2* to these responses. That knocking down *elt-2* expression did not impair general intestinal expression and did not affect animal survival in a panel of stress assays supports the notion that in adult animals, ELT-2 contributes to survival as an immune regulatory mechanism. Nevertheless, considering the many responding genes that lack a promoter GATA motif, ELT-2 is probably not the sole regulator of worm infection responses.

There are similarities and differences in the role we identified for ELT-2 in regulating innate immune responses to that previously described for the *Drosophila* GATA transcription factor, Serpent. Like ELT-2, Serpent is essential for the development of the gut. However, Serpent contributes to early endodermal development (14), whereas ELT-2 is responsible for terminal differentiation of intestinal cells. Serpent is additionally required for the formation of the mesodermal fat body and hematopoiesis. With regard to regulating innate immune responses, Serpent functions in hemocytes and in larval fat body to induce the expression of antimicrobial peptides in response to systemic infection (15). Whether Serpent controls epithelial responses in larvae or adults is still not known, but it was reported as dispensable for antimicrobial peptide induction in epithelial cells of the embryonic epidermis (42). In contrast to Serpent's role in systemic responses, ELT-2 responds to local infection and regulates an essentially local response in epithelial cells. This comparison suggests the two genes are not orthologs. Instead, we propose the *Drosophila* endoderm-specific *dGATAe* transcription factor, which is required for terminal differentiation of fruitfly intestinal cells and has greater sequence similarity to ELT-2 (33), as a likely *elt-2* ortholog.

In human immune responses, GATA transcription factors (GATA1-3) function in hematopoietic cell-terminal differentiation (39). Our identification of ELT-2 as a regulator of local innate immune responses, through sequence homology and tissue specificity, pointed at another branch of this family, the endodermal GATA transcription factors GATA4-6, as possible functional homologs. Our experiments in human lung epithelial cells suggest that the function we identified for ELT-2 in *C. elegans* is conserved, consistent with a role for GATA6 in protective local epithelial responses in the lung. It is yet unknown what genes are regulated by GATA6 during infection. One such target previously reported is

surfactant protein A (43). The high prevalence of GATA motifs in promoters of epithelially expressed genes responding to infection suggests that additional targets exist. Our results further encourage exploring the function of GATA transcription factors in the lungs of cystic fibrosis patients, who are most affected by *P. aeruginosa* infections.

Materials and Methods

Infection. Worms were infected by feeding on bacteria grown on modified nematode growth (NG) plates at 25°C (18).

Growing Worms for Microarray Experiments. Synchronized *C. elegans* cultures were prepared by using standard techniques (*Supporting Text*). Young adult animals were split between modified NG plates preseeded with OP50 or PA14 and incubated for 4, 12, or 24 h before harvesting. This experiment was repeated three times on different occasions.

Microarray Analyses. cDNA probes were prepared from poly $(A)^+$ RNA and hybridized to spotted microarrays (*Supporting Text*). Log-transformed (base 2) ratios were used for all statistical analyses.

Identifying Genes That Respond to Infection. The data set that passed quality filters contained 7,972 PCR products representing 7,308 genes (Dataset 1, which is published as supporting information on the PNAS web site). We identified infection-response genes with a procedure based on two-way ANOVA, which allows estimation of false discovery rates (FDR; *Supporting Text*). This procedure was implemented in MATLAB and is available upon request. To maximize the number of true positives, we selected an FDR of 20% (Fig. 8, which is published as supporting information on the PNAS web site).

RNAi Knockdown and Survival Assays. To avoid confounding effects of progeny in survival assays, we used the sterile worm strains *spe-26(it112)* and *glp-4(bn2);rrf-3(pk1426)*. Additionally, we used wild-type animals of the N2 strain, made sterile by exposure to RNAi directed against *cdc-25.1*, a gene important for germline proliferation (44): gravid worms were laid on *cdc-25.1* RNAi plates for 4 h and then transferred to similar plates for an additional 4 h of egg laying. After that, gravids were removed, and eggs were left to hatch and grow in the presence of *cdc-25.1* RNAi, developing into sterile adult animals.

Larval stage L4 (*spe-26* or *glp-4;rrf-3* mutants) or young adult (N2 *cdc-25.1*-sterilized) worms were fed for 48 h with double-strand (ds)RNA-expressing *E. coli* (*Supporting Text*) and then transferred to preseeded PA14 plates. Bacteria containing an empty RNAi expression vector served as control. Results of survival assays were analyzed using Kaplan–Meier analysis, scoring for significance with the log-rank test.

Lifespan, heat-stress, and cadmium-survival assays were performed with kanamycin- or UV-killed OP50–1 bacteria (*Supporting Text*).

qRT-PCR. qRT-PCR was performed using standard techniques (*Supporting Text*). Each amplification reaction was performed in duplicate or triplicate, and fold changes were normalized to changes in actin RNA levels. *t* tests were performed using normalized threshold-cycle values. Quantification of GATA6 RNA levels in A549 cells was performed similarly, using RNA levels of the human actin gene *ACTB* for normalization.

Cell Culture, siRNA Transfection, RNA Extraction, and Cytotoxicity Assays. A549 human lung epithelial cells (American Type Culture Collection, Manassas, VA) were grown under standard conditions until confluent, transferred to six-well plates, and transfected with *GATA6* or *p105* siRNA SMARTpools using the DharmaFECT1 reagent per the manufacturer's instructions (Dharmacon, Lafayette, CO). Forty-eight hours after siRNA treatment, cells were washed, incubated for 1 h in serum-free medium, and infected with log-phase PBS washed PA14 in a multiplicity of infection of 100:1. Three hours or 1 h after infection, respectively, cells were either harvested for RNA extraction (RNeasy; Qiagen, Valencia, CA) or assayed for cytotoxic effects using Trypan blue (Sigma–Aldrich, St. Louis, MO).

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