Title: Low pH caused ROS reduction of the intestine defect the innate immunity in *C. elegans*

Preview for " Saida Benomar *et. al.* 2020. The C. elegans CHP1 homolog, pbo-1, functions in innate immunity by regulating the pH of the intestinal lumen"

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Introduction

The innate immune system is the first protection mechanism to defense when the body undergo microbial invasion or tissue injury. An important function of the innate immune system is to create an unfavorable chemical environment for microbial growth. In human, the stomach plays important role in killing or inactivating pathogens. It can maintain the acid condition by secreting the hydrochloric acid which is deleterious to many microbial pathogens. This process helps to make sure that the pathogens will be eliminated before they are passed into the intestinal track[1]. Besides manipulating extracellular pH at a low level, the digestive system can also protect body from pathogen by producing reactive oxygen species (ROS). In *C. elegans*, the researchers demonstrated that the dual oxidase Ce-Duox1/BLI-3 expressed in worm intestine can generate ROS induced by infection[2]. Furthermore, the generation of ROS by oxidase in the barrier epithelium is a highly conserved innate immune defense mechanism. For example, in mammalian system, NADPH oxidase 1 (Nox1) and dual oxidase 2 (Duox2) which are highly expressed in the colon, are shown to play a potential role in innate immune response and host defense[3].

The acidic extracellular environment can trigger cellular signaling events either through the cell surface or through changes in intracellular environment, and therefore represent as an endogenous danger alerting signal to the innate immunity[4]. However, the acidification in the extracellular region does not always benefit the host. In cystic fibrosis patients, the acidification occurs in the airway surface liquid via the proton pumping by H+/K+ adenosine triphosphatase (ATP12A) actually impairs the host defenses against infection[5]. Hence, maintaining and balancing extracellular pH level are critical in innate immunity. However, little attention has been paid so far to analyze how the extracellular pH is regulated on the immune response.

C. elegans is an excellent model for studying innate immune function because its lack of an adaptive immune system eliminates many confounding variables. *C. elegans* dynamically controls its intestinal pH, but it is unclear how this contributes to pathogen defense. In this study, Saida Benomar *et. al.* utilizing *C. elegans* as the model system to illustrate how intestinal pH plays role in innate immunity.

Result

Brian D. Ackley's group used a pH sensitive dye, KR35, to monitor the pH change in the intestine of *C. elegans*. They found out that there is an acidic wave, also called pH oscillation. It starts with posterior fluoresce transition to the anterior region of the intestine, and stays at the anterior region for about 3-7 seconds, which termed the Maximum Anterior Transition (MAT). This process is coordinated with the defecation motor program (DMP), in which the protons are released at the basolateral membrane of the intestine by the Na+/H+ exchanger PBO-4. Their previous results showed that mutations in *pbo-4* fail to produce the MAT during pH oscillations because it has a Posterior body contraction absent phenotype (Pbo), which showed a defect in contracting posterior muscles during DMP[6].

To investigate the relationship between MAT and Pbo phenotype, they examined another Pbo mutant, *pbo-1*(sa7). *pbo-1* mutants didn't show any pH oscillations but had anterior acidity, which is opposite of the *pbo-4* phenotype. *pbo-1; pbo-4* double mutants had pH oscillations and more neutral intestinal pH compared with *pbo-1* single mutants, which shared more similarity to *pbo-4* mutants. It suggests that the anterior acidity in *pbo-1* mutants is dependent on pbo-4 function, and PBO-1 can function as a negative regulator. The authors were also interested in other physiological consequences caused by acidic wave of the intestine. They hypothesized that low intestinal pH could protect the animal from pathogens entering the alimentary canal. To test this notion, they fed N2, *pbo-1*, *pbo-4*, and *pbo-1; pbo-4* mutants with the pathogen *E. faecalis, S. aureus, P. aeruginosa, and* non-panthgenic *E. coli.* The result indicates that *pbo-1* mutants were more susceptible, while *pbo-4* mutants were not significantly more susceptible than N2 in survival and motality experiments. *pbo-1* mutants also showed no increase in the pathogen load compared with wild type. In addition, the lifespan of wild type*, pbo-1*, and *pbo-4* mutants were similar, which suggests that *pbo-1* mutants had no defect in lifespan. Taken together, pbo-1 might be functioning as a part of the innate immune system, responsible for protecting the animal from pathogens.

To test whether the acidic intestinal pH contributes to pathogen susceptibility of the *pbo-1* mutant, the authors use sodium bicarbonate in the infection plates as a physiologic buffer for acid in the alimentary canal. The results showed that bicarbonate treatment reduced the susceptibility in *pbo-1* mutants, but did not provide wild type or *pbo-4* mutants any additional resistance during *E. faecalis* exposure. Thus, the increased susceptibility to pathogens was, at least partially, due to the increased acidity of the alimentary canal of *pbo-1* animals, which indicates that intestinal pH change might be part of the response to pathogens.

To test this idea, they compared intestinal acidity of the wild-type *C. elegans* fed with E coli or pathogenic *E. faecalis* or *P. aeruginosa* along with KR35 dye. The result showed KR35 fluorescence was reduced in animals fed with pathogens, suggesting the intestinal pH was more neutral in pathogen-fed animals. They also used KR54 to verify this result. To get higher resolution data on this phenomenon, they used laser-scanning confocal microscopy to visualize KR35 fluorescence in *E. coli*, *E. faecalis* and *P. aeruginosa* fed wild-type and *pbo-1* mutant animals. The results confirmed that part of the normal response to infection is to neutralize the intestinal pH.

They then hypothesized that pbo-1 or infection-dependent changes in pH could regulate the production of H2O2 because recent work has identified that the production of H2O2 functions as a part of innate immune system when C. elegans undergoes infection[2]. They used Amplex Red assay to measure the amounts of H2O2 produced in response to infection. They found out that pbo-1 mutants are defective for production of H2O2, which can be compensated by supplying bicarbonate. This indicates that the reduction of H2O2 production in the pbo-1 mutant is pH-dependent.

Significance and Future direction

In this study, the authors utilized acid-activated fluorophore KR35 to characterize the dynamic pH changes in worm intestine. They showed that pbo-1 mutants have more acidic intestinal pH and more pathogen susceptibility compared to wild-type animals. In addition, pbo-1 mutants are defective on production of ROS which might contribute to increased susceptibility to pathogens. The treatment of bicarbonate and removing PBO-4 both suppressed the altered phenotypes observed in pbo-1 mutants. Overall, this study is significant because it shows that PBO-1 related pH changes in worm intestine upon pathogens infection play vital roles in innate immunity. Furthermore, it illustrates that intestinal pH can directly affect the production of H2O2 which

is important for pathogen defense. However, this paper still leaves several interesting questions for future researches:

The KR35 fluorophore showed the overall trend of pH alteration in pbo-1 mutant intestine, which is decreased compared to wild type, but what the exact change of pH upon pathogen infection which is essential for the activities of intestinal digesting enzymes and secreted oxidase/peroxidase is still unknown.

What is importance of PBO-1 in mediating anterior to posterior pH wave? Does it have different expression levels between anterior and posterior intestine cells?

The pbo-4 mutant has an opposite effect on pH in the intestine from that of pbo-1 mutant, and the pbo-1; pbo-4 double mutant is more similar to the pbo-4 single mutant. These suggest that PBO-1 might be the negative regulator of PBO-4 to mediate intestinal pH. But the detailed mechanism underlying it is still elusive. Does PBO-1 regulated PBO-4 directly or through other indirect pathways? What would be the phenotypes if they overexpress PBO-4 in pbo-1 mutant?

Is the affected activity of dual oxidase BLI-3 in pbo-1 mutant due to the altered intestinal pH or the direct regulation by PBO-1 or PBO-4? Are the activities of other oxidases and peroxidases also affected in pbo-1 mutants? In addition, it remains unknown whether the production or stability of H2O2 is altered in pbo-1 mutants.

Reference

1. Smith, J.L. (2003). The role of gastric acid in preventing foodborne disease and how bacteria overcome acid conditions. J Food Prot *66*, 1292-1303.
2. Chavez, V., Mohri-Shiomi, A., and Garsin, D.A. (2009). Ce-Duox1/BLI-3 generates reactive oxygen species as a protective innate immune mechanism in Caenorhabditis elegans. Infect Immun *77*, 4983-4989.
3. Rokutan, K., Kawahara, T., Kuwano, Y., Tominaga, K., Sekiyama, A., and Teshima-Kondo, S. (2006). NADPH oxidases in the gastrointestinal tract: a potential role of Nox1 in innate immune response and carcinogenesis. Antioxid Redox Signal *8*, 1573-1582.
4. Rajamaki, K., Nordstrom, T., Nurmi, K., Akerman, K.E., Kovanen, P.T., Oorni, K., and Eklund, K.K. (2013). Extracellular acidosis is a novel danger signal alerting innate immunity via the NLRP3 inflammasome. J Biol Chem *288*, 13410-13419.
5. Shah, V.S., Meyerholz, D.K., Tang, X.X., Reznikov, L., Abou Alaiwa, M., Ernst, S.E., Karp, P.H., Wohlford-Lenane, C.L., Heilmann, K.P., Leidinger, M.R., et al. (2016). Airway acidification initiates host defense abnormalities in cystic fibrosis mice. Science *351*, 503-507.
6. Beg, A.A., Ernstrom, G.G., Nix, P., Davis, M.W., and Jorgensen, E.M. (2008). Protons act as a transmitter for muscle contraction in C. elegans. Cell *132*, 149-160.