COMMENTARY

Ethanolamine: A Signal to Commence a Host-Associated Lifestyle?

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ABSTRACT **Ethanolamine (EA) is a compound prevalent in the gastrointestinal (GI) environment. The ability to catabolize this compound has been associated with important GI pathogens, including enterohemorrhagic *Escherichia coli* O157:H7 (EHEC). It has been hypothesized that the ability of EHEC to utilize EA as a source of nitrogen provides EHEC with an important competitive advantage in the gut. However, new work by Kendall et al. (mBio 3:e00050-12, 2012) suggests that the role of EA in EHEC pathogenesis may be more fundamental; EA appears to be a signal for EHEC to commence its virulence program. In this commentary, I review the previously described connections of EA to bacterial pathogenesis in the GI tract, highlight the important findings of this new study, and note areas where further research is needed to fully comprehend the connection of EA utilization to bacterial pathogenesis.**

The GI (gastrointestinal) environment is rich in cell membrane lipid components due to the constant turnover of the mucosal epithelium as well as the gut-associated microbiota. EA is a breakdown product of the membrane phospholipid phosphatidylethanolamine (PE) and can be used as a source of nitrogen and sometimes carbon by bacteria capable of catabolizing this compound. EA is broken down into acetaldehyde and ammonia by the two-subunit ammonia lyase EutBC (reviewed in reference 1). A recent analysis showed that some species contain only the *eutBC* genes, whereas others have a complex cohort of *eut* (ethanolamine utilization) genes encoding many accessory functions, such as the enzymatic ability to further break down acetaldehyde (*eutD*, -*E*, and -*G*), structural components to build a specialized *eut* microcompartment (*eutS*, -*K*, -*L*, -*M*, and -*N*), and regulatory factors (*eutR*, -*V*, and -*W*). The gut-associated bacterial species that contain the genes necessary for EA breakdown include both Gram-positive and Gram-negative genera, such as *Clostridium*, *Listeria*, *Enterococcus*, *Escherichia*, and *Salmonella* (2). These bacteria are pathogens of the gut, with the exception of *Escherichia coli* and *Enterococcus faecalis*. Only particular strains of *E. coli*, such as enterohemorrhagic *E. coli* O157:H7 (EHEC) and enteropathogenic *E. coli* (EPEC), are harmful in the intestinal environment, while *E. faecalis* is thought to be a normal constituent of the microbiota and is sometimes used as a probiotic (3).

Overall, there appears to be a global association of the EA degradation pathway with GI pathogens, as noted by a study that used literature mining and genomic analysis to predict factors linked with bacterial food poisoning (4). Hence, EA utilization is a possible virulence determinant, and many specific examples in which it plays this role can be found in the literature. For example, the global virulence regulators CsrA and Fis, found in *Salmonella enterica* subsp. *enterica* serovar Typhimurium, were also shown to regulate *eut* gene expression (5, 6), and mutations in some of the *eut* genes led to a loss of virulence in a mouse model (7). The *eut* genes were also found to be strongly upregulated in the intestines of mice infected by *Listeria monocytogenes* (8), correlating to a role in pathogenesis. While *E. faecalis* is a normal commensal of the mammalian gut microbiota, it causes a deadly infection in the intestine of the model organism *Caenorhabditis elegans*. A *eut* mutant was attenuated in its ability to kill the nematode, again suggesting an association between EA utilization and virulence (9).

For the pathogens EHEC and EPEC, several links between ethanolamine metabolism and virulence have been noted. Over a decade ago, it was observed that EHEC and EPEC preferentially bind PE over the common membrane phospholipid phosphatidylcholine (PC) (10). The same researchers later showed that the apoptosis induced by EHEC and EPEC augments the amount of PE in the outer leaflet of the membrane, increasing EHEC attachment (11). Further studies, using just EPEC, discovered that the pathogen changes host phospholipid metabolism to induce this increase in outer-leaflet PE (12). In addition to PE's role in promoting the binding and destruction of host cells by pathogenic *E. coli*, it could be argued that this pathogen-induced increase in the amount of host cell PE could serve as a valuable source of EA. Recent data demonstrated that EA is degraded and serves as a source of nitrogen (but not carbon) for EHEC under conditions that mimic the intestinal environment. EHEC grown in bovine intestinal contents (BIC) displayed increased expression of the *eut* gene cluster. The EA content of the BIC was measured and shown to decrease with time, upon incubation with EHEC. EA utilization by EHEC gave it a competitive growth advantage over *eut* mutant strains, and it was shown to catabolize EA much more avidly than commensal *E. coli* strains (13).

In total, these data suggest that EA utilization bestows some sort of competitive advantage on intestinal pathogens that are metabolically capable of its degradation, but the mechanism is not understood for any of the species mentioned. Because ethanolamine can serve as a source of nitrogen and, in some species, carbon, the "nutrition hypothesis" has most frequently been offered (1). However, in new work by Kendall et al. on EHEC (14), a role for EA beyond simply a source of nutrients is postulated. Their data suggest that EA may serve as a critical signal that tells the bacterium that it is in the intestinal environment, triggering the appropriate gene expression program, which in the case of EHEC is virulence gene expression (14).

In their study, Kendall et al. (14) demonstrated that growth in

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minimal medium containing EA as a nitrogen source increased the expression of genes important for virulence. These include *stx2a* (encoding Shiga toxin), genes encoding parts of the LEE type III secretion system, and genes encoding important virulence regulators (*ler*, *qseC*, and *qseE*). The *eut* genes were also induced in the medium containing ethanolamine, but interestingly, the observed expression of the virulence genes was not dependent on the *eut* catabolic enzymes; upregulation was observed in a *eutB* mutant. However, expression of the virulence genes was at least partially dependent on the positive transcriptional regulator of the *eut* locus, EutR, and influenced by the growth conditions (14).

Under aerobic growth conditions, expression of *qseC* and *qseE* was dependent on EutR. The situation was more complicated for *ler* and *stx2a*. During early log phase, there were high levels of expression of these genes in the absence of *eutR*, with and without EA, while in the wild-type background, high levels of expression were dependent on the presence of EA. These data suggest that EutR has a repressive effect on *ler* and *stx2a* expression during early log phase. In mid-log to late log phase, high levels of *ler* and *stx2a* expression were observed in the *eutR* background, dependent on the presence of EA, while the presence of EA did not affect expression much in the wild-type background. This could reflect the fact that EA was metabolized in the wild-type background by mid-log to late log phase and was no longer present, whereas it was not significantly metabolized and its levels did not decrease in cultures of the *eutR* mutant. However, the most significant aspect of these data was that EA still regulated expression of some of the virulence genes in the *eutR* mutant background. This strongly suggests the involvement of a second, currently unidentified regulator which, like EutR, senses the presence of EA (14).

Kendall et al. (14) also looked at the effects of EA and EutR on virulence gene expression under anaerobic conditions, which are more reflective of the intestinal environment. Because metabolism of EA did not appear to be necessary for virulence gene expression, they also examined whether small amounts of EA, in the micromolar range, could still induce expression. Under these conditions, all the virulence genes examined were induced in the presence of EA. Almost all concentrations of EA increased expression, with two peaks being apparent, one in the micromolar range and one in the millimolar range. The two peaks are again suggestive of two sensors being involved. Both basal and EA-induced expression was lower in the *eutR* background than the wild type. However, EA-dependent induction was still observed in the *eutR* mutant for all the virulence genes except *ler*, again suggestive of another EA-sensing regulator. *eutR* expression was induced only by millimolar concentrations of EA, suggesting that the postulated second component senses the micromolar concentrations of EA (14).

EA's ability to induce expression of important EHEC virulence genes leads to the prediction that EHEC asserts a more virulent phenotype in the presence of this compound. To test this model with host cells, Kendall et al. (14) looked at the formation of attaching-and-effacing (AE) lesions by EHEC on epithelial cells in the presence of EA. Lesion formation was significantly enhanced in the presence of micromolar concentrations of EA. Loss of *eutR* inhibited EA-induced lesion formation, but there were still significantly more lesions in the presence of EA than in its absence. In total, these results support a role for EA as a signal for EHEC's virulence program that is sensed in part by the regulatory protein EutR and in part by an independent, currently unidentified component (14).

Since EA is present in large quantities in the intestine, it makes biological sense that this compound is one of the major signals that initiates EHEC's virulence program. However, an understanding of how this signal is sensed and relayed requires further elucidation. From the data, EutR plays a role, but it is clearly not the only sensing component involved; loss of EutR does not completely abolish the response to EA. It is also not exactly understood how EutR is activated by EA. Genetic studies carried out in *S*. Typhimurium suggest that direct binding of EA activates EutR (15), but biochemical evidence is lacking. It will also be important to determine whether the influence on virulence gene expression by EutR and the unidentified regulator is direct or indirect.

A larger question is whether detection of EA is a general mechanism by which bacteria sense the intestinal and possibly other host-associated environments. A related question is whether this sensing is dependent on the organism's possessing EutR. While *Enterobacteriaceae*, such as *E. coli* and *S*. Typhimurium, sense EA and regulate the *eut* genes with EutR, the *Firmicutes*, including *L. monocytogenes* and *E. faecalis*, accomplish sensing and regulation via a two-component system consisting of EutW (EA-sensing histidine kinase) and EutV (response regulator) (reviewed in reference 1). It will be interesting to see if the EutVW regulatory system also controls gene expression associated with an intestinal lifestyle. Also, the lifestyle may not necessarily be pathogenic. As mentioned above, *E. faecalis* is a normal constituent of the mammalian GI tract, and it is possible that EA signals the maintenance of a commensal-associated gene expression program.

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