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Campylobacter jejuni Host Tissue Tropism: A Consequence of Its Low-Carb Lifestyle?

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Abstract

Mechanisms underlying virulence properties of *Campylobacter jejuni* have historically been difficult to identify. In this issue of *Cell Host & Microbe*, Hofreuter et al. (2008) show that *C. jejuni*'s ability to metabolize glutamine, glutathione, and asparagine affects its ability to colonize specific host tissues. These findings reflect the emerging theme of bacterial physiology directly impacting pathogenesis.

Campylobacter jejuni is the leading cause of bacterial-induced acute gastroenteritis in the developed world, yet the precise mechanisms by which it causes disease remain unclear (Young et al., 2007). Although it causes severe human disease, *C. jejuni* harmlessly colonizes many animal species (i.e., birds and cows) and is typically acquired through ingestion of contaminated poultry, milk, or water. Symptoms of campylobacteriosis range from mild, watery diarrhea to severe bloody diarrhea resembling dysentery. *C. jejuni* can also invade and transcytose host cells, and strains that are highly invasive also tend to cause more severe disease. In rare instances, infection with *C. jejuni* can lead to more serious medical sequelae, including the ascending bilateral paralysis Guillain-Barré Syndrome. In contrast to other, better-understood enteric pathogens such as *Salmonella*, *Shigella*, and *E. coli* spp., *C. jejuni* is quite fastidious, with a complex metabolism requiring microaerophilic and capnophilic growth conditions. Furthermore, *C. jejuni* is asaccharolytic, lacking the capacity both to transport and catabolize most carbohydrates, and instead relies on amino acids such as serine, glutamate, and aspartate as primary carbon and energy sources (Kelly, 2008).

Also in perplexing contrast to other enteric pathogens, *C. jejuni* lacks many "classic" virulence factors, such as exotoxins and type III secretion systems for injecting effector proteins into host cells. Even the eagerly anticipated release of the first sequenced *C. jejuni* genome (strain 11168) in 2000 failed to identify any obvious means by which the organism causes disease (Parkhill et al., 2000). However, the sequence did provide significant insight into *C. jejuni* biology, including the identification of several metabolic pathways, protein glycosylation systems and a polysaccharide capsule, and mechanisms underlying its high rate of phase variation. The more recent sequencing of additional strains, including one known to be highly virulent and invasive (strain 81-176), likewise did not uncover new virulence factors (Fouts et al., 2005; Hofreuter et al., 2006; Pearson et al., 2007). While this was surprising and initially somewhat disappointing, these comparative genomics efforts did show that the majority of strain-specific differences occurred in metabolic genes, suggesting their potential importance in *C. jejuni* pathogenesis.

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In this issue, Hofreuter et al. demonstrate that two such variant genes, both of which encode enzymes involved in amino acid metabolism, play key roles in tissue tropism of the virulent and invasive *C. jejuni* strain 81-176 during host infection (Hofreuter et al., 2008).

The first enzyme, γ-glutamyltranspeptidase (GGT), was identified as present in the genome of strain 81-176 but not strain 11168 (Hofreuter et al., 2006). In that study, an 81-176 strain deleted for GGT was used to demonstrate that GGT is required for mouse intestinal colonization following oral infection. Similar findings using a chick infection model and a different GGT-harboring *C. jejuni* strain have also been reported (Barnes et al., 2007). GGT catalyzes the conversion of glutathione and glutamine to glutamate. Hofreuter et al. now clearly demonstrate that the ability of multiple *C. jejuni* strains and clinical isolates to utilize glutamine or glutathione as a sole carbon source absolutely depends on the presence of GGT (Hofreuter et al., 2008). They further demonstrate that this occurs via periplasmic conversion of glutamine or glutathione to glutamate, which is subsequently transported into the cytoplasm via the Peb1A glutamate/aspartate binding protein. Interestingly, GGT was dispensable for mouse liver colonization of strain 81-176 following intraperitoneal infection, suggesting that the ability of *C. jejuni* to disseminate systemically does not require glutamine or glutathione utilization.

Conversely, this study also demonstrates that a periplasmic isoform of asparaginase (AnsB) is dispensable for intestinal colonization but is important for optimal liver colonization of strain 81-176. The authors elegantly show that this is due not only to the presence of *ansB*, but specifically to the presence of an alternative *ansB* start codon and downstream secretion signal sequence (SS) found in strain 81-176 but not strain 11168. The SS confers periplasmic localization to AnsB; using a series of strains containing *ansB* isoforms harboring or missing the SS, 81-176, and 11168 strains complemented with each other's *ansB* genes, and a survey of numerous clinical isolates, Hofreuter et al. also clearly show that periplasmic AnsB is required for *C. jejuni* to utilize asparagine as a sole carbon source. As above, this occurs via deamination of asparagine to aspartate, followed by Peb1A-dependent aspartate transport to the cytoplasm.

This work collectively suggests that *C. jejuni* utilizes glutamine and/or glutathione for intestinal but not liver colonization, and asparagine for liver but not intestinal colonization, defining roles in specific tissue tropism for specific nutrient utilization genes. These findings are particularly relevant for a pathogen such as *C. jejuni* given its lack of hallmark virulence factors and reliance on amino acids as carbon sources. The thorough molecular genetic analyses undertaken to demonstrate these points, especially the AnsB SS requirement, are commendable, especially in an organism that is still much more difficult to manipulate genetically than most other enteric pathogens.

The periplasmic AnsB and GGT observations presented in this study may also have direct implications for other Gram-negative pathogens, including ε-proteobacterial species closely related to *C. jejuni*. SignalP analyses indicate that periplasmic AnsB asparaginases occur in other bacteria that can localize to the liver. One of these organisms is the hepatotropic ε-proteobacterium *Helicobacter hepaticus.* In contrast, all sequenced genomes of the stomach-restricted pathogen *Helicobacter pylori* encode only a predicted cytoplasmic asparaginase. Periplasmic AnsB proteins are likewise predicted in the genomes of *E. coli, Salmonella, Shigella, Yersinia, Francisella,* and *Klebsiella* spp., each of which can disseminate to the liver, but not in bacteria such as *Vibrio cholerae* or *Neisseria gonorrhoeae,* which are primarily restricted to other body compartments.

In contrast, GGT may be more important for pathogens that colonize mucosal tissues. BLAST analyses indicate that GGT is not found in *H. hepaticus* but is present in all sequenced *H. pylori* strains; indeed, *H. pylori* GGT activity has been shown to be important for gastric

colonization as well as for uptake of glutathione and glutamine from the extracellular environment (McGovern et al., 2001; Shibayama et al., 2007). GGT is also found in most strains of nearly all of the other abovementioned Gram-negative pathogens.

The above In silico analyses together with the data presented in Hofreuter et al., lend themselves to several intriguing interpretations. For instance, while tissue tropism is clearly multifactorial, the potential role of periplasmic AnsB and asparagine metabolism in pathogen persistence in the liver and possibly dissemination to other organs is an exciting notion to consider. The presence of GGT in numerous mucosal pathogens may also be consistent with defined roles for mammalian GGT, glutamine, and glutathione in maintaining mucosal tissue health. Glutamine and glutathione in particular are known to exert protective effects involving both the innate and adaptive immune systems. Both *H. pylori* and *C. jejuni* GGT have been implicated as inducing host cell apoptosis (Barnes et al., 2007; Shibayama et al., 2007); one ensuing hypothesis is that this may in part be due to glutamine or glutathione “theft” from the gastric and intestinal mucosa. Future work exploring potential roles in other pathogens for asparagine utilization in liver dissemination and glutamine and/or glutathione utilization in mucosal damage may shed light on these hypotheses.

This study also raises several intriguing questions specific to *C. jejuni* pathogenesis. First, the paradox of *C. jejuni*'s prevalence in the environment given its fastidious nature is a key question in *C. jejuni* research, and it will be interesting to establish if GGT and/or periplasmic AnsB participate in growth or survival during lower-nutrient conditions as encountered in water, milk, and other non-in vivo environments. Second, although a potential role for GGT in host cell invasion and intracellular survival has been described (Barnes et al., 2007), this has not been explored for periplasmic AnsB. Invasion and intracellular survival are typically associated with virulence, and a correlation between them, AnsB, and liver dissemination would provide additional mechanistic insight into *C. jejuni* virulence properties. Finally, is there a direct correlation between GGT and periplasmic AnsB with *C. jejuni* disease? All of the clinical strains surveyed in this study for presence or absence of GGT and the AnsB SS, including 11168, were originally human isolates and thus presumably capable of causing diarrhea. Although this information is not currently available (J. Galan, personal communication), it would be very interesting to know if these strains are also associated with watery versus bloody diarrhea or other clinical differences. Along similar lines, it will be important to establish intestinal and liver colonization properties of clinical strains not harboring GGT or the AnsB SS. This will allow better insight into whether the observations described represent a general trend or are specific to the strain investigated; if the latter, it will also be interesting to explore other means by which strains that cannot utilize glutamine, glutathione, and/or asparagine have evolved to establish tissue tropism.

In summary, this work both provides key new insight into *C. jejuni* pathogenesis and highlights the underappreciated but emerging theme of the importance of physiology and basic metabolic processes in both pathogen tropism and pathogenesis in general. Examples of this connection have been published for other pathogens, but as with *C. jejuni*, historically have not been as well acknowledged in terms of understanding virulence as have studies of more traditional virulence factors. The direct connection between tissue tropism and specific nutrient utilization demonstrated here provides clear proof that metabolism and pathogenesis are intimately interconnected; further research into these areas should yield additional new angles linking these aspects of pathogen biology.

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