Gut Microbes 1:1, 70-72; January/February 2010; © 2010 Landes Bioscience

Salmonella's iron armor for battling the host and its microbiota

Manuela Raffatellu[1] and Andreas J. Bäumler[2,*]

[1]Department of Microbiology and Molecular Genetics; University of California at Irvine; Irvine, CA USA; [2]Department of Medical Microbiology and Immunology; University of California at Davis; Davis, CA USA

Most *Salmonella enterica* serotypes are associated with acute intestinal inflammation and diarrhea in humans. While the mechanisms triggering intestinal inflammation are well studied, relatively little is known about how the pathogen benefits from causing disease. Recent work has provided first insights into the genetic design that enables *S. enterica* to benefit from the host response by outgrowing the microbiota in the gut. The pathogen gained an edge over its competitors by acquiring genes conferring resistance against antimicrobials, such as lipocalin-2, that are encountered in the intestinal lumen only during inflammation. This strategy enables the pathogen to exploit host responses to gain a competitive advantage over other microbes during its growth in the inflamed gut.

Key words: gastroenteritis, lipocalin-2, siderophore, salmonella, inflammation

Submitted: 06/10/09

Accepted: 09/03/09

Previously published online: www.landesbioscience.com/journals/gutmicrobes/article/10951

*Correspondence to: Andreas J. Bäumler; Email: ajbaumler@ucdavis.edu

Addendum to: Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio S-P, Paixao TA, et al. Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. Cell Host Microbe 2009; 5:476–86; PMID: 19454351; DOI: 10.1016/j.chom.2009.03.011.

The importance of bacterial pathogens for human health has traditionally focused research efforts on elucidating the mechanisms by which these agents cause host responses leading to disease. Results from these investigations have provided invaluable insights into the pathogenesis of many infections. However, studies on pathogenic mechanisms rarely reveal how the microbe benefits from causing a particular sign of disease in its host. In case of pathogens that reside in a human or animal reservoir, the main evolutionary pressures arise from the necessity to transmit from an infected to a naïve host. To fully understand the biology of a pathogen, it is therefore important to elucidate whether its ability to cause disease provides a benefit by increasing its transmission success, thereby providing direct insights into how virulence evolved in this bacterial species.

Recent research on the pathogenesis of *Salmonella enterica* infections provides a rare glimpse into why this organism evolved to cause acute intestinal inflammation. The vast majority of the more than 2,000 known *S. enterica* serotypes are associated with a localized gastroenteritis in immunocompetent individuals. Thus, the ability to cause gastroenteritis likely represents a primitive trait already present in a common ancestor of the *S. enterica* lineage. What sets *S. enterica* apart from its non-pathogenic close relatives, such as commensal *Escherichia coli*, is its ability to invade the intestinal mucosa and survive in macrophages in the underlying tissue. These pathogenic properties are conferred by the invasion associated type III secretion system (T3SS-1) and a second type III secetion system (T3SS-2), which mediates macrophage survival. T3SS-1 and T3SS-2 are encoded by Salmonella pathogenicity island (SPI) 1 and SPI2, respectively, two large DNA regions that are present in all members of *S. enterica* but absent from commensal *E. coli*.[1] Mutational inactivation of T3SS-1 and T3SS-2 dramatically reduces the ability of *S. enterica* serotype Typhimurium to elicit intestinal inflammation.[2] While it is clear that the functions of T3SS-1 and T3SS-2 are instrumental for the pathogenesis of gastroenteritis, identification of these virulence factors did not explain how *S. enterica* may profit from causing disease.

A significant conceptual advance came though the observation that intestinal inflammatory responses elicited by T3SS-1 and T3SS-2 provide a selective advantage by promoting outgrowth of *S. enterica* serotype Typhimurium in the intestinal lumen,[3] thereby enhancing fecal

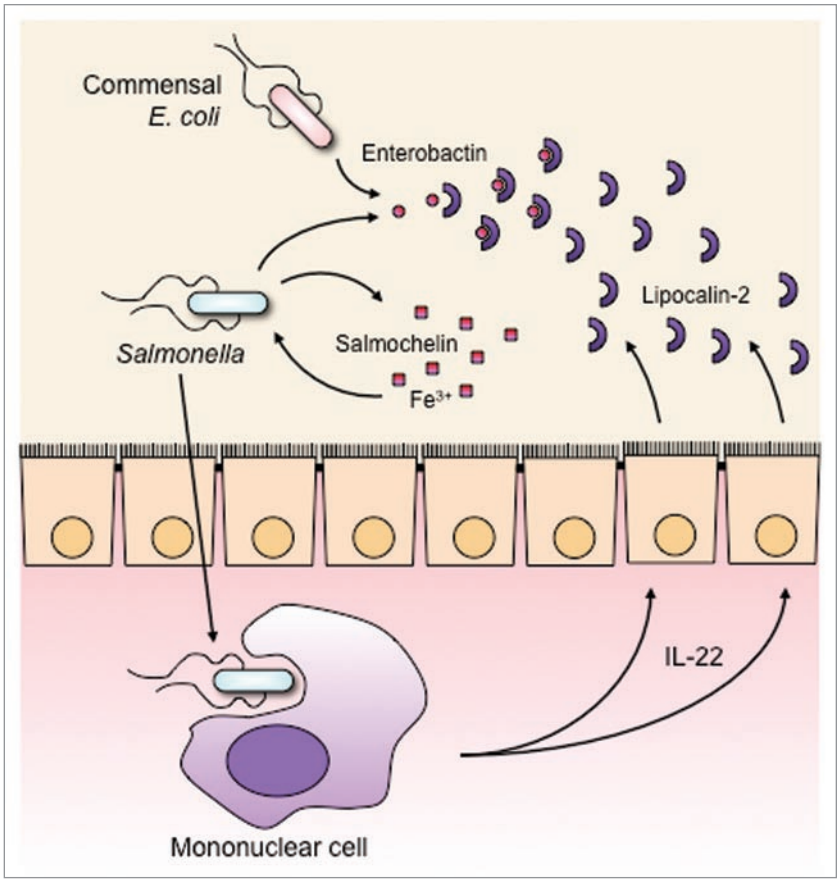

Figure 1. Iron acquisition of *S. enterica* serotype Typhimurium (Salmonella) in the inflamed intestine. *S. enterica* serotype Typhimurium triggers intestinal inflammation by invading epithelial cells and surviving in mononuclear phagocytes (i.e., macrophages and dendritic cells). The resulting production of IL-22 triggers the production of epithelial derived lipocalin-2. Lipocalin-2 prevents bacterial iron acquisition by binding the siderophore enterochelin, which is produced by both *S. enterica* serotype Typhimurium and commensal *E. coli*. By producing salmochelin, a siderophore not bound by lipocalin-2, *S. enterica* serotype Typhimurium can benefit from antimicrobial responses encountered in the intestinal lumen by outgrowing competing intestinal microbes.

oral transmission.[4] This strategy does not directly benefit the population of invading bacteria, which trigger inflammation and are eventually cleared from intestinal tissue by the ensuing immune response. However, a second population of *S. enterica* serotype Typhimurium resides in the intestinal lumen and can take advantage of intestinal inflammation, as indicated by its ability to outgrow the resident microbiota.[5] It has been proposed that one of the mechanisms that may enable *S. enterica* serotype Typhimurium to outgrow the gut microbiota during inflammation is its resistance to antimicrobials encountered in the gut lumen.[6] During evolution of this inflammation-adapted pathogenic lifestyle, horizontal gene transfer of the pathogenicity islands enabling *S. enterica* to trigger inflammation (i.e., SPI1 and SPI2) had to be accompanied by acquisition of genetic material that conferred resistance to the ensuing antimicrobial responses in the intestinal lumen. The first such DNA region to be identified is the *iroN iroBCDE* gene cluster.

The *iroN iroBCDE* gene cluster is present in all members of *S. enterica*, but absent from commensal *E. coli*, suggesting that this DNA region was acquired by the Salmonella lineage at a similar time as SPI1 and SPI2.[7-9] The *iroN* gene encodes an outer membrane receptor for the uptake of low molecular weight iron-chelators, termed siderophores, indicating a role in bacterial iron acquisition.[10] Based on its phylogentic distribution it has been proposed that the *iroN iroBCDE* gene cluster may confer a function that sets *S. enterica* apart from commensal *E. coli*.[9] However, revealing the identity of this trait required a number of key findings about the battle for iron that unfolds during infection. The main siderophore produced by *E. coli* and *S. enterica* is enterobactin, also known as enterochelin, a cyclic trimer of 1,2-dihydroxybenzoylserine.[11] While it is long known that enterobactin enables *E. coli* and *S. enterica* to remove iron from high affinity host binding proteins, such as transferrin, in vitro,[12] it remained an enigma why this siderophore could not supply bacteria with iron during an infection. The answer turned out to be an antimicrobial host protein, termed lipocalin-2, which specifically binds enterobactin,[13] thereby preventing its use for bacterial iron acquisition.[14] Subsequent work revealed that the *iroN iroBCDE* gene cluster confers lipocalin-2 resistance[15] by mediating the biosynthesis (IroB),[16] export (IroC)[17] and uptake (IroN) of salmochelin, a linear glycosylated derivative of enterobactin.[18] Salmochelin is no longer bound by lipocalin-2 and thus promotes iron acquisition during infection.[17] These findings set the stage for determining the role of the *iroN iroBCDE* gene cluster during life in the inflamed intestine.

The entry of *S. enterica* serotype Typhimurium into intestinal tissue triggers a massive cytokine storm, including high expression levels of interleukin (IL)-22.[19,20] IL-22 stimulates epithelial cells to release substantial amounts of lipocalin-2 into the intestinal lumen,[21] suggesting that resistance to this antimicrobial host protein may confer a specific benefit during growth in the inflamed intestine. Indeed, the presence of an intact IroN outer membrane salmochelin receptor provided a benefit during growth of *S. enterica* serotype Typhimurium in the lumen of the inflamed intestine, but not during growth in the normal intestine.[21] Collectively, these data suggest that acquisition of the *iroN iroBCDE* gene cluster by horizontal gene transfer was one of the genetic changes that enabled *S. enterica* to take advantage of T3SS-1/T3SS-2 mediated intestinal inflammation by enhancing its ability to compete with the microbiota in the gut lumen. This study provides further insight into the genetic design that enables

S. enterica to outgrow the microbiota by using the host response to transform the normal intestine into a more hostile niche, in which the pathogen gains an edge over its competitors.

While commensal *E. coli* colonize the surface of the normal intestine and are lipocalin-2 sensitive, uropathogenic *E. coli* are adapted to growth in the inflamed bladder. Consistent with the idea that lipocalin-2 resistance is required for colonization of inflamed mucosal surfaces, uropathogenic *E. coli* carry the *iroN iroBCDE* gene cluster[22] and require these genes for urovirulence.[23] Lipocalin-2 also contributes to host defenses encountered on other inflamed mucosal surfaces, such as the nasal mucosa,[24] the oral cavity,[25] the gastric mucosa[26] and the lung.[27] Lipocalin-2 resistance may thus be a prerequisite for colonizing inflamed mucosal surfaces in general.

Acknowledgements

We would like to thank Sebastian E. Winter for helpful comments on the manuscript. Work in M.R.'s lab is supported by Public Health Service Grant AI083619 and the IDSA ERF/NIFID Astellas Young Investigator Award. Work in A.J.B.'s laboratory is supported by Public Health Service grants AI040124, AI044170, AI076246 and AI079173.

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