Salmonella "Sops" Up a Preferred Electron Receptor in the Inflamed Intestine

James B. Bliska and Adrianus W. M. van der Velden

Department of Molecular Genetics and Microbiology and Center for Infectious Diseases, Stony Brook University, Stony Brook, New York, USA

ABSTRACT **The microbiota of the mammalian intestinal tract represents a formidable barrier to colonization by pathogens. To overcome this resistance to colonization, bacterial pathogens use virulence factors to induce intestinal inflammation, which liberates nutrients for selective use by the infecting microbe. Studies of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) infection in a streptomycin-treated mouse colitis model show how virulence factor-induced inflammation can produce nutrients used selectively by the pathogen. Type III secreted effectors of invading *S.* Typhimurium induce inflammation in the intestine (epithelial cells and lamina propria macrophages) that causes changes in the composition of the lumen. For example, neutrophils entering the intestine produce superoxide, resulting in production of tetrathionate, which *S.* Typhimurium in the lumen uses as an electron acceptor for anaerobic respiration. In their recent study, Lopez et al. demonstrate that *S.* Typhimurium strains that are lysogenized with a phage encoding type III effector SopE induce the host to produce nitric oxide synthetase (iNOS) in the intestine (C. A. Lopez et al., mBio 3:e00143-12, 2012). Nitric oxide is converted to a highly favorable electron acceptor, nitrate. As a result, growth of *sopE*$^+$ *S.* Typhimurium in the intestine lumen is boosted by nitrate respiration. This is a striking example of how acquisition of a virulence factor by horizontal gene transfer can increase the metabolic fitness of a pathogen. Interestingly, survival of the invading bacteria is probably decreased as a result of the SopE-induced immune response, and yet the *S.* Typhimurium bacteria that multiply in the lumen of the intestine can efficiently disseminate to another host, ensuring success for the pathogen.**

One of the most important functions of the microbiota of the mammalian intestine is to promote resistance to colonization by pathogens (1). Recent studies show that enteric bacterial pathogens induce inflammation to overcome resistance to colonization (2, 3). Nutrients and cofactors produced during inflammation can be selectively utilized by enteric pathogens to grow in the intestinal lumen. These new findings highlight the concept that pathogens evolved virulence mechanisms to allow access to host nutrients (4).

Insights into how pathogens induce inflammation to overcome colonization resistance have come from the study of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), which causes acute intestinal inflammation and diarrhea. *S.* Typhimurium invades enterocytes and colonizes lamina propria macrophages, inducing inflammatory responses. Invasion, colonization, and induction of inflammation by *S.* Typhimurium require the function of two type III secretion systems, T3SS-1 and T3SS-2. T3SS-1 and T3SS-2 are encoded within *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2), respectively. These systems deliver effector proteins into host cells to trigger specific responses. The resulting inflammatory responses, including the epithelial transmigration of neutrophils, cause changes in the availability of cofactors and nutrients that allow bacteria remaining in the intestinal lumen to outcompete the microbiota (3). Thus, although the invading population of bacteria may effectively find themselves at a "dead end," the inflammatory responses that they induce promote growth of the luminal population of *S.* Typhimurium (5, 6). High densities or "blooms" of *S.* Typhimurium in the intestine facilitate transmission and are thus critical for its success as a pathogen (7). Growth of *S.* Typhimurium may be accompanied by blooms of other enterobacteria in the inflamed intestine (8).

The mostly anaerobic environment of the gut lumen is maintained by the scavenging of oxygen by the microbiota. The majority of the microbiota are strict anaerobes (e.g., *Bacteroides* and *Clostridium* species) that rely on fermentation of amino acids and complex carbohydrates for energy production. A major fermentation by-product, hydrogen sulfide (H_2S), is detoxified by intestinal epithelial cells, which convert it to thiosulfate ($S_2O_3^{2-}$). Interestingly, it appears that *S.* Typhimurium can take advantage of $S_2O_3^{2-}$, as part of its strategy for overcoming colonization resistance (9). This and other recent findings have come from the use of a murine colitis model that relies on pretreatment of mice with the antibiotic streptomycin (10). This antibiotic treatment reduces the number of bacterial microbiota by greater than 10-fold, providing *S.* Typhimurium with a "foothold" that is important for induction of colitis. Using this model, it was shown that reactive oxygen species (ROS) released by neutrophils in the intestinal lumen convert $S_2O_3^{2-}$ to tetrathionate ($S_4O_6^{2-}$) (9). *S.* Typhimurium contains a gene cluster, *ttrSR ttrBCA*, which allows utilization of $S_4O_6^{2-}$ as an electron acceptor for anaerobic respiration. The phospholipid phosphatidylethanolamine is also abundant in the inflamed intestine and can be utilized by *S.* Typhimurium as a carbon source for anaerobic respiration in conjunction with $S_4O_6^{2-}$ as the electron acceptor (11). Remarkably, the *ttrSR ttrBCA* gene cluster is carried on SPI-2, showing a direct linkage between genes required for virulence (T3SS-2) and those required for metabolism (*ttrSR ttrBCA*).

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Address correspondence to James B. Bliska, jbliska@ms.cc.sunysb.edu.

S. Typhimurium utilizes T3SS-1 to inject the effector SopE into enterocytes to trigger invasion. SopE is a guanine nucleotide exchange factor (GEF) that activates Rho GTPases CDC42 and Rac1. SopE is also of major importance for eliciting intestinal inflammation (6, 10, 12). Analysis of SopE function in the mouse colitis model revealed that inflammatory responses result from the activation of caspase-1 in enterocytes (12). Cleavage and secretion of the cytokines interleukin-1β (IL-1β) and IL-18 appear to be an important cause of the inflammatory response downstream of caspase-1 activation (12). Interestingly, *sopE* is carried on a bacteriophage that has lysogenized a small fraction of *S.* Typhimurium isolates (13). A multidrug-resistant clone of *S.* Typhimurium that was responsible for an epidemic in humans and cattle in Europe in the 1980s is lysogenized with the *sopE* phage. This phage is also present in laboratory *S.* Typhimurium strain SL1344. Although SopE does increase the intensity of intestinal inflammation (12), it has remained unclear how acquisition of the *sopE* phage enhanced the fitness of *S.* Typhimurium.

In their recent study, Lopez et al. provide the first insight into how SopE increases the fitness of *S.* Typhimurium (14). The initial key observation was that the *sopE*$^+$ SL1344 strain was present at higher levels in the intestines of mice than was an isogenic *sopE* mutant at day 4 postinfection. In addition, the presence of *sopE* was associated with increased expression of inducible nitric oxide synthetase (iNOS) in the intestine at 3 days postinfection. The product of iNOS, nitric oxide (NO), can react with ROS produced by neutrophils to form peroxynitrite ($ONOO^-$). Peroxynitrite can in turn isomerize to form nitrate (NO^{3-}), which is a much-preferred electron acceptor compared to tetrathionate. The growth advantage of the *sopE*$^+$ *S.* Typhimurium strain at day 4 postinfection was dependent upon nitrate respiration. This was shown using a mutant strain of *S.* Typhimurium in which the three operons required for nitrate respiration (*nar* and *nap* operons) were inactivated by mutation. Nitrate respiration-dependent luminal growth of *S.* Typhimurium was lost in iNOS-deficient mice. Interestingly, nitrate negatively regulated expression of the *ttr* operons, which explains why a *ttr* mutant *sopE*$^+$ strain was not outcompeted by the wild-type *S.* Typhimurium. In hindsight, one realizes that the initial discovery of the importance of tetrathionate respiration by Winter et al. (9) may be due to the fact that the *S.* Typhimurium strain used (IR715) was not lysogenized with the *sopE* phage.

Although the data from the work of Lopez et al. (14) clearly show that SopE boosts growth of *S.* Typhimurium through nitrate respiration in the inflamed intestine, many additional details of this process remain to be fleshed out. For example, the connection between SopE function and activation of iNOS expression is undefined. The authors suggest that SopE activates caspase-1, which in turn cleaves IL-18 to form the mature cytokine. Secretion of mature IL-18 could induce production of gamma interferon (IFN-γ), which in turn would act as a potent inducer of iNOS production. While this scenario is likely correct, it remains to be determined how SopE activates caspase-1 (12), and the cellular sources of IFN-γ and iNOS in the intestine are undefined.

Do other enteric pathogens benefit from inflammation-induced nitrate respiration? There are some hints that the answer is yes. Nitrate respiration has been shown to increase colonization of the chicken cecum by *Campylobacter jejuni* (14). A number of intestinal pathogens, including *Shigella* species, enteropathogenic *Escherichia coli*, and *Citrobacter* species inject effectors with GEF activity (the WxxxE family of GEF effectors) into host enterocytes, raising the possibility that gut inflammation during these infections could result in part from effector-triggered caspase 1 activation (15). Thus, it is reasonable to assume that the caspase-1–iNOS–nitrate axis is operating in many cases of pathogen-induced intestinal inflammation.

In addition to enhancing growth of *sopE*$^+$ *S.* Typhimurium in the inflamed intestine, nitrate respiration likely has other important biological outcomes. One of the more interesting outcomes is that *S.* Typhimurium-inflicted enteropathy allows for parallel blooms of the pathogen and of resident commensal *Escherichia coli*. Stecher et al. (8) showed that these blooms facilitated conjugation of the colicin plasmid p2 from *sopE*$^+$ *S.* Typhimurium to *E. coli in vivo*. This horizontal gene transfer was possible because the inflammatory response raised numbers of both donor and acceptor bacteria to values >100-fold above those typically encountered in the normal mammalian intestine.

It is also interesting to consider the cost to *S.* Typhimurium associated with SopE-induced host immune responses. Activation of caspase-1 is considered to be host protective against systemic infection by *S.* Typhimurium. This can be seen in the work of Muller et al. (12), where significantly fewer *sopE*$^+$ *S.* Typhimurium organisms were recovered from the mesenteric lymph nodes of caspase-$1^{+/+}$ mice than from those of caspase-$1^{-/-}$ mice. In this context, SopE activity could be considered a "pattern of pathogenesis" (16), i.e., a virulence factor activity that triggers a protective innate immune response. Patterns of pathogenesis may allow the innate immune system to "sense" the virulence potential of a pathogen and to adjust the immune response to a level appropriate to the challenge (16). Several general types of activities may be recognized as patterns of pathogenesis, including pathogen growth, cytosolic access (e.g., access mediated by specialized toxin secretion systems), or disruption of the actin cytoskeleton (16); the last might be the case with SopE.

If one focuses on the cost associated with SopE activity on the "dead-end" population of bacteria invading the lamina propria, it is difficult to understand the selective advantage for *S.* Typhimurium to maintain this pattern of pathogenesis. However, if one considers the "big picture," i.e., that SopE promotes growth and most likely dissemination of the bacteria remaining in the lumen of the intestine, it is easy to see that there is a selective advantage for *S.* Typhimurium to exploit innate immunity using a pattern of pathogenesis to trigger mucosal inflammation.

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