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Short Article

The *Bacillus subtilis* Quorum-Sensing Molecule CSF Contributes to Intestinal Homeostasis via OCTN2, a Host Cell Membrane Transporter

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SUMMARY

Bacteria use quorum-sensing molecules (QSMs) to communicate within as well as across species. However, the effects of QSMs on eukaryotic host cells have received limited attention. We report that the quorum-sensing pentapeptide, competence and sporulation factor (CSF), of the Gram-positive bacterium *Bacillus subtilis* activates key survival pathways, including p38 MAP kinase and protein kinase B (Akt), in intestinal epithelial cells. CSF also induces cytoprotective heat shock proteins (Hsps), which prevent oxidant-induced intestinal epithelial cell injury and loss of barrier function. These effects of CSF depend on its uptake by an apical membrane organic cation transporter-2 (OCTN2). Thus, OCTN2-mediated CSF transport serves as an example of a host-bacterial interaction that allows the host to monitor and respond to changes in the behavior or composition of colonic flora.

INTRODUCTION

The enteric microbiota is a unique ecological niche where microorganisms live normally in the digestive tract in a balanced relationship with other species and the host. The relationship is complex and incompletely understood, often involving bidirectional signals and interactions that not only influence the behavior of microflora but also host responses essential to the maintenance of intestinal homeostasis (Rakoff-Nahoum and Medzhitov, 2006). Prime examples of the latter are the host toll-like receptors (TLR) that continuously monitor luminal microbial pattern molecules that are essential for regulation of innate immune responses and epithelial cytoprotection (Pasare and Medzhitov, 2005). Among bacteria, population dynamics are influenced by the secretion of numerous metabolites and effector molecules that promote species stability, adaptation, and survival within this environment. Quorum sensing is perhaps the most elegant of these processes, providing bacteria with the ability to communicate and change behavior of the same or other species in response to conditions and perturbations of the environment (Bassler and Losick, 2006 and Camilli and Bassler, 2006). Both Gram-positive and Gram-negative organisms utilize quorum-sensing molecules (QSMs), which in the former are usually bioactive peptides, whereas in the latter include nonpeptide molecules such as acyl-homoserine lactone (Bassler and Losick, 2006). Because they play a role in determining the diversity and composition of the enteric microbiome, the profile of QSMs at any given time reflects the status or impending changes in the microbiota. Whether eukaryote cells have the ability to detect the complex array of QSMs has not been well studied, but such an ability would allow the host to appropriately respond to physiological or pathophysiological perturbations in the microbiota. Investigations of a QSM, N-(3-oxododecanoyl)-L-homoserine lactone, secreted by *Pseudomonas aeruginosa* have demonstrated activation of the p38 mitogen-activated protein kinase as well as phosphorylation of the eukaryotic translation initiation factor eIF2α in macrophages and bronchial epithelial cells (Vikstrom et al., 2005; Kravchenko et al., 2006). This lactone also alters tight junctional permeability of cultured intestinal cell monolayers (Vikstrom et al., 2006). The present studies add to these observations in demonstrating that the pentapeptide QSM, ERGMT, also known as CSF (competence and sporulation factor) from *Bacillus subtilis*, is transported into mammalian intestinal epithelia through a novel cell membrane transporter, organic cation transporter isotype 2 (OCTN2). *Bacillus subtilis* is a well-characterized, obligate, Gram-positive aerobe that is prevalent in the environment. It is reported to be part of avian, mammalian, and human enteric flora, although its prevalence in the latter in unknown (Benno et al., 1989; Rhee et al., 2004; Barbosa et al., 2005; Tam et al., 2006).

Numerous reports have suggested that *B. subtilis* has health-beneficial properties and has potential as a probiotic agent (Casula and Cutting, 2002; Alexopoulos et al., 2004; D'Arienzo et al., 2006). Once taken up by intestinal epithelial cells, CSF activates key survival pathways including p38 MAP kinase and protein kinase B (Akt) and also induces cytoprotective heat shock proteins (Hsps), the latter preventing oxidant-induced intestinal epithelial cell injury and loss of barrier function.

RESULTS

Bacillus subtilis-Produced CSF Induces Hsps and Activates the Protein Kinase B/AKT Survival and p38 MAP Kinase Stress Signal Pathways in Caco2$_{bbe}$ Cells

Heat shock proteins (Hsp) are essential for maintenance of intestinal homeostasis (Rakoff-Nahoum et al., 2004; Pasare and Medzhitov, 2005; Rakoff-Nahoum and Medzhitov, 2006), rendering colonic epithelial cells less susceptible to injury and stress (Arvans et al., 2004). Intestinal epithelia express many heat shock proteins, including inducible Hsp27(human)/Hsp25(murine) and Hsp70 and constitutively expressed heat shock cognate Hsc70. The nomenclature, Hsp27 and Hsp25, defines the related small heat shock proteins of approximate molecular weight of 27 kDa and 25 kDa of human and murine cells, respectively (Morimoto, 1993 and Morimoto, 2002). Physiological expression of inducible heat shock proteins like Hsp27/25 and Hsp70 is maintained by microbial-derived molecules, including pattern recognition ligands (Rakoff-Nahoum et al., 2004), accounting for their predominant expression in surface colonocytes (see Figure S1 in the Supplemental Data available with this article online). Therefore, we assessed the effects of conditioned media (CM) from several representative strains of enteric bacteria in inducing heat shock protein 27 (Hsp27) expression in human colonic epithelial Caco2$_{bbe}$ cells. Some of the strains, *Bacillus subtilis*, *Bifidobacterium breve*, *Lactobacillus rhamnosus GG (ATCC53103)*, *E. coli Nissle*, and *Lactobacillus plantarum* were chosen as all have been used in probiotic formulations. Other strains, such as *Enterobacter aerogenes* and *Proteus mirabilis*, were studied because of their association with human colonic flora (Hooper et al., 2001; Eckburg et al., 2005). As shown in Figure 1A, of the strains that were tested, most Gram-positive, but not Gram-negative, bacteria significantly induced Hsp27 expression in Caco2$_{bbe}$ cells (results expressed as a percent of control Hsp27 response to thermal stress, 41.5°C × 23 min). In light of this dichotomous response of Gram-positive bacteria, we considered the possibility that secreted agents such as quorum-sensing peptides might be mediating the actions of this group of microbes.

B. subtilis was selected for further study because it is a well-characterized soil and water saprophyte but is also found in enteric flora of many species including humans. It also has known probiotic activity (Solomon et al., 1996; Kunst et al., 1997; Lazazzera et al., 1997; Levin and Grossman, 1998; Tam et al., 2006). A specific strain, *B. subtilis* JH642, was selected as a number of deletions of specific genes have been made from this wild-type strain, making possible identification of potential bioactive factors (Solomon et al., 1996; Levin and Grossman, 1998). Conditioned medium from the *B. subtilus* strain JH 642 increased Hsp27 expression to nearly the same extent as heat shock (Figure 1B), whereas neither experimental condition altered expression of the constitutively expressed heat shock cognate Hsc70. The latter was anticipated because Hsc70 in most cells is quite stable and less influenced by exogenous stimuli or cell stress. This insures that Hsc70 continues to function in critical processes such as protein folding, chaperone function, and in formation and stabilization of protein complexes (Morimoto, 1993 and Morimoto, 2002). To further characterize the factor(s) that induced Hsp27, CM from JH 642 was size-separated by a 3 kDa molecular mass cutoff filter with bioactivity largely remaining in the filtrate indicating a small molecular mass (Figure 1C). Additionally, the Hsp27-inducing bioactivity was heat stable and pepsin sensitive.

B. subtilis produces and secretes many bioactive agents, but its CSF, a QSM, fits the parameters of the above physiochemical characteristics. CSF is a cationic pentapeptide corresponding to the C-terminal 5 amino acids of the 40 amino acid polypeptide encoded by the *phrC gene* (Kunst et al., 1997) and functions in quorum sensing (Lazazzera et al., 1997) with a physiological concentration range between 10–100 nM to alter Bacillus population behavior in pure culture (Solomon et al., 1996). To assess CSF's potential biological role in colonic epithelial cells, CM from wild-type (JH 642) and the CSF-deficient JH 642-derived *B. subtilis* strain, RSM 121, were added to Caco2$_{bbe}$ cells. CM derived from RSM121 (delta CSF) failed to induce Hsp27 in Caco2$_{bbe}$ cells, implicating CSF in this effect (Figure 1D). To further evaluate this possibility, CSF (ERGMT) was chemically synthesized and purified. CSF induced Hsp27 in Caco2$_{bbe}$ cells (top western blot of Figure 1E), and this induction is a concentration-dependent fashion which is physiologically relevant for *B. subtilis* cultures (Figure S2). Although the concentration of CSF or any other bacterial QSMs in intestinal luminal contents has not been determined, the same concentration range that affects *Bacillus* also stimulates the cultured human intestinal epithelial cells. Additionally, we tested the activation of other signaling pathways involved in cell survival and found that CSF activated the Akt and p38 MAPK pathways (lower two sets of western blots in Figure 1E). In intestinal epithelial cells, the former has been shown to be important in promoting Hsp25 expression (Tao et al., 2006) and the latter in blocking apoptosis by inhibiting caspase-3 after polyamine depletion (Zhang et al., 2004). In contrast, two other pathways, JNK and ERK, were not influenced by CSF (data not shown). As a control, a scrambled pentapeptide, EMTRG, did not induce Hsp27 or activate the Akt and p38 MAPK pathways (data not shown).

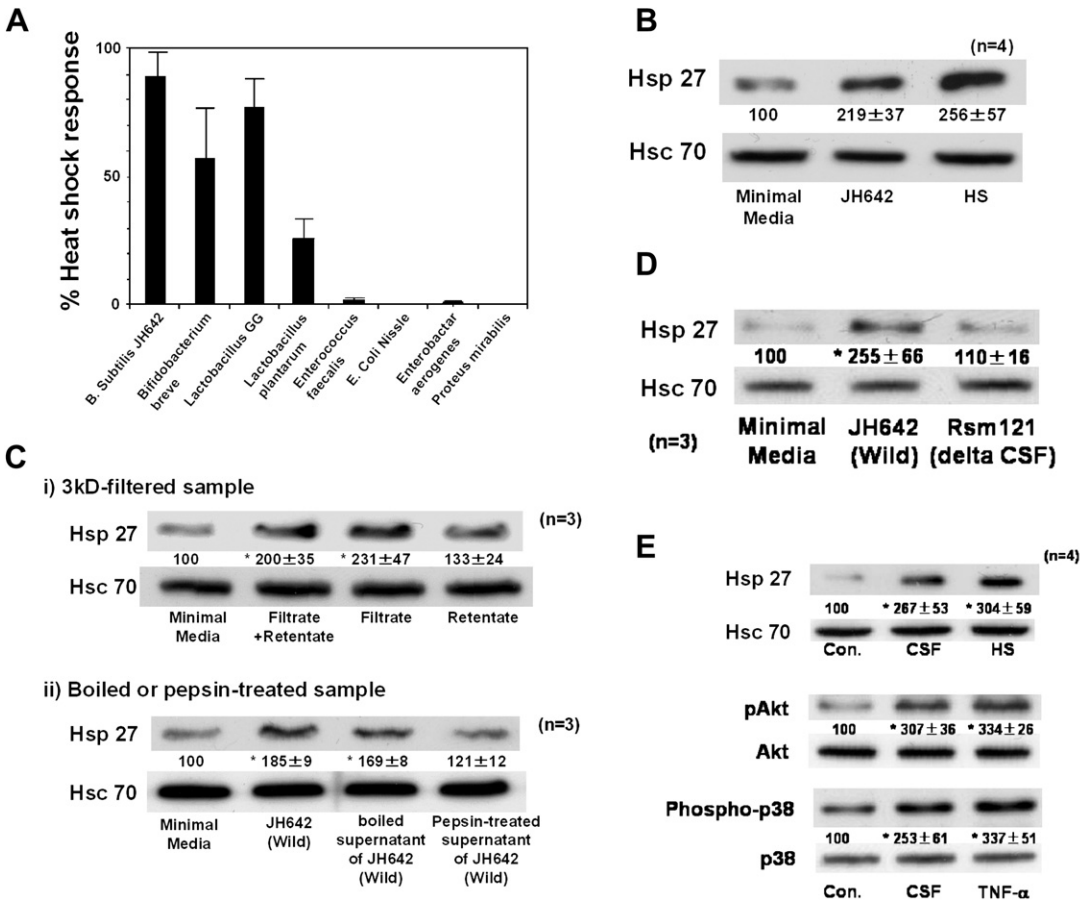

Figure 1. Bioactive Agents in Conditioned Media from Representative Gram-Positive Bacteria, Including the Quorum-Sensing Molecule CSF of *Bacillus subtilis*, Induce Heat Shock Protein 27 in Colonic Caco2$_{bbe}$ Cells

(A) Conditioned media from most Gram-positive (left 5 bars), but not Gram-negative (right 3 bars), bacterial species significantly induced Hsp27 protein expression in Caco2$_{bbe}$ cells. Densitometry results are expressed as a percent of the Hsp27 response induced by heat shock in paired control cells.

(B) Secreted factors in *B. subtilis* (JH 642, wild-type) conditioned media induce Hsp27. Densitometry mean ± SEM of western blots are normalized to unstimulated control values (arbitrarily set at 100 units). No changes in the protein expression of the heat shock cognate, Hsc70, were observed in any of the experimental conditions, including heat shock (HS).

(C) Bioactive factors from *B. subtilis* (JH 642, wild-type) that induce Hsp27 (shown by western blot) are less than 3 kDa (top set of panels), heat stable, and pepsin sensitive (lower set of panels). Filtrate and retentate were prepared by passing CM through a 3 kDa Centricon filter.

(D) CM from wild-type *B. subtilis* JH642, but not CSF-deficient Rsm121 (delta CSF), induces Hsp27 (western blot).

(E) CSF (100 nM) also stimulates phosphorylation of Akt and p38 MAPK (shown in western blots), two additional survival pathways of intestinal epithelial cells. Responses to HS and TNF-α (100 ng/ml) stimulation are shown as positive controls. Heat shock cognate, Hsc70, was used as a loading and experimental control. "Con." indicates control cells that were not treated with peptides. HS control samples are shown, obtained from cells two hrs after transient exposure to 41.5°C × 23 min. *$p < 0.05$ compared with control at the same time point by analysis of variance.

OCTN2 Transports CSF and Mediates CSF Effect on Hsps Induction in Caco2$_{bbe}$ Cells

CSF-mediated activation of an early competence promoter (*srfA*) (Lazazzera et al., 1997; Levin and Grossman, 1998) in *B. subtilis* cells is dependent on the uptake by a Bacillus oligopeptide transporter. Could a convergent mechanism develop in eukaryotic host that would mediate a specific uptake of bacterial QSM peptides? In fact, other peptides, such as bacterial chemotactic peptides, can be transported by eukaryote apical membrane oligopeptidyl transporters (Charrier et al., 2006). We specifically focused on the apical membrane organic cation transporter, OCTN2, as a candidate for CSF-uptake because of its transport preference for substrates having physiochemical properties close to CSF (e.g., cationic oligopeptide) (Tamai et al., 2000 and Peltekova et al., 2004). OCTN2 is believed to be the main transporter for dietary carnitine, but its abundant expression in the colon is unexplained, as most carnitine is absorbed in the small intestine (Tamai et al., 2000 and Ohashi et al., 2001). OCTN2 is primarily expressed by surface epithelial cells of the colon that are in direct contact with the luminal contents and microbes and which exhibit sustained expression of microbial-induced heat shock proteins (Rakoff-Nahoum

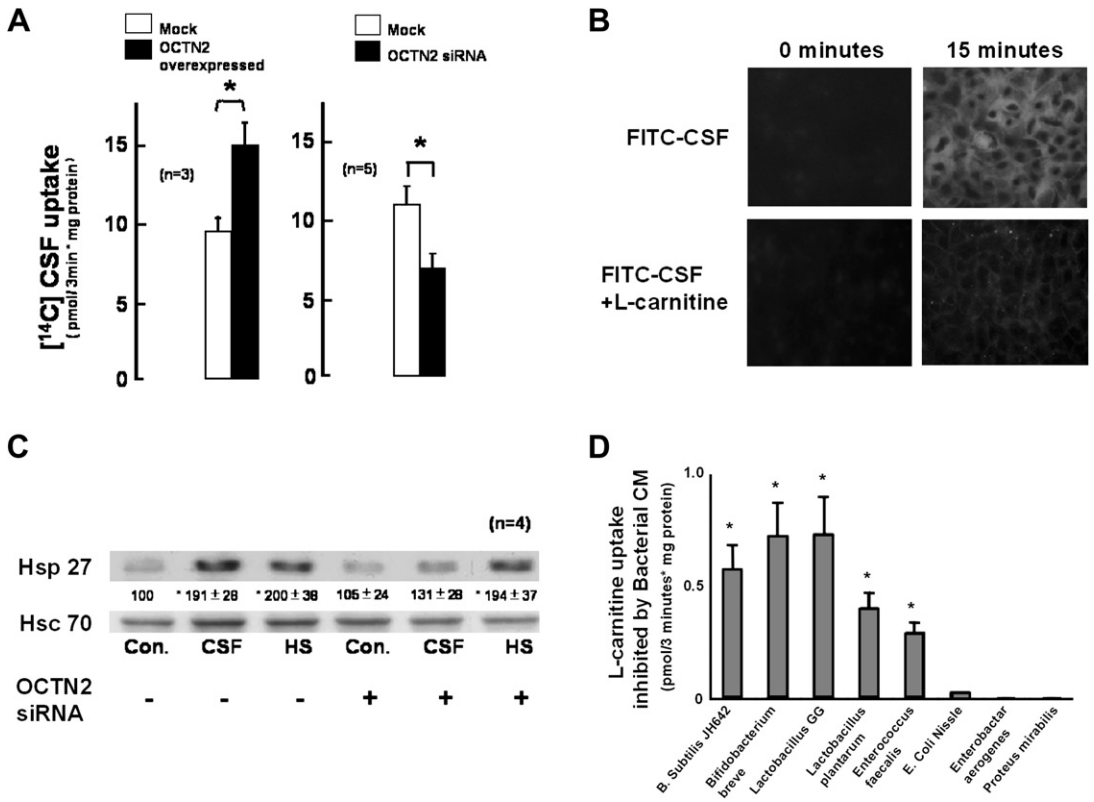

Figure 2. OCTN2-Mediated Transport of CSF Is Pivotal in Hsp27 Induction

(A) Caco2$_{bbe}$ uptake of ^{14}C-labeled CSF (competence and sporulation factor) is enhanced in OCTN2-overexpressed cells and less in OCTN2-siRNA treated cells (left panel). *p < 0.05 compared to mock transfected control cells.

(B) Caco2$_{bbe}$ cells take up FITC-labeled CSF which distributes in the cytoplasm (15 min incubation), an effect competed by 10 mM L-carnitine (lower panels).

(C) CSF-induced Hsp27 (shown by western blot) is blocked in cells treated with OCTN2-siRNA (without effect on Hsc70 or heat shock [HS] response). Control (Con) cells were not stimulated with CSF. HS, samples obtained from cells two hrs after transient exposure to 41.5°C × 23 min.

(D) Effects of CM of Gram-positive (leftmost 5 bars) and Gram-negative bacteria (3 right bars) on OCTN2 transport, assessed by competition of [^{3}H]-L-carnitine uptake by confluent human colonic epithelial Caco2$_{bbe}$ monolayers. L-carnitine uptake is used to functionally characterize OCTN2 transport activity (Tamai et al., 2000 and Ohashi et al., 2001). Values represent the amount of [^{3}H]-L-carnitine uptake reduced by bacterial CM. Data are means ± SEM for the number of experiments indicated. *p < 0.05 compared with control at the same time point by analysis of variance.

et al., 2004) (Figure S1). OCTN2, in contrast to OCTN1, is also expressed in Caco2$_{bbe}$ cells (Figure S3A).

As shown in Figure 2A, ^{14}C-labeled CSF was readily taken up by Caco2$_{bbe}$ cells, an effect that was increased in OCTN2-transfected cells and inhibited in cells with siRNA-silenced OCTN2 expression (Figures S3B and S4C). Similar, but more pronounced, effects were observed in OCTN2-transfected human fibroblast HSWP cells that normally exhibit minimal endogenous expression of OCTN2 (Figures S4A and S4B). FITC-labeled CSF was also rapidly taken up by Caco2$_{bbe}$ cells (Figure 2B) and distributed throughout the cytosol within 15 min, an effect competed by L-carnitine (10 mM) implicating OCTN2 transport. In addition, while CSF competed with L-carnitine uptake, a scrambled pentapeptide for CSF (EMTRG) did not, suggesting the specificity of OCTN-mediated transport of the peptide (Figure S4C). Taken together, these studies provide compelling evidence for uptake of CSF by OCTN2. CSF induction of Hsp27 was also blocked by inhibiting OCTN2 expression with siRNA, whereas Hsp27 induction by heat shock (41.5°C × 23 min) was not affected (Figure 2C), showing that OCTN2 is required for CSF to mediate Hsp27 induction. As shown in Figure 2D, conditioned media of other Gram-positive, in contrast to Gram-negative, bacteria also appear to compete with Na-dependent, L-carnitine uptake of Caco2$_{bbe}$ cells, suggesting OCTN2 uptake of soluble molecules derived from these organisms.

OCTN2-Mediated CSF Uptake Protects Epithelial Cells from Oxidant Stress

To assess the functional role of CSF and OCTN2 in epithelial homeostasis, we examined whether CSF protects epithelial cells against oxidant (NH_2Cl, monochloramine)-induced injury using ^{51}Cr release. Pretreatment of Caco2$_{bbe}$ monolayers with conditioned medium from wild-type JH642 CM protected cells against oxidant-induced injury, whereas pretreatment with CM

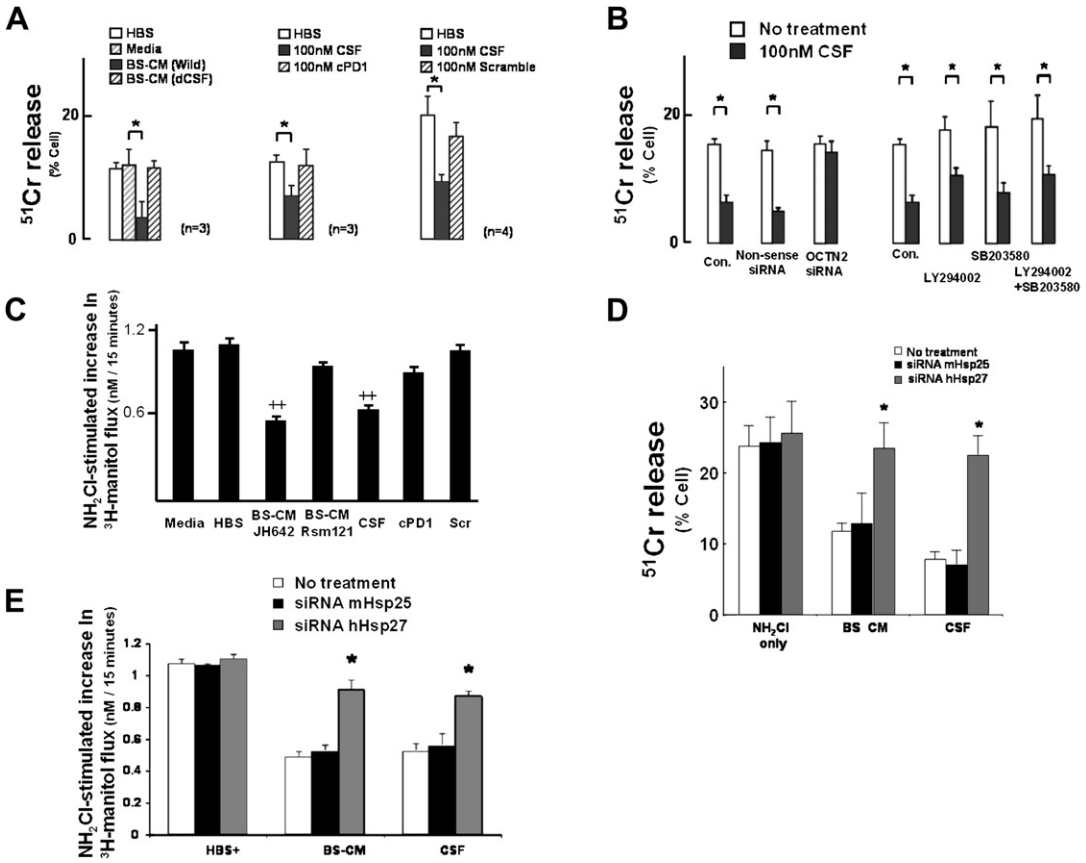

Figure 3. Condition Media of *B. subtilis* and Quorum-Sensing CSF Protect Intestinal Epithelial Cells against Oxidant-Induced Cell Death and Loss of Barrier Function

(A) Pretreatment of $Caco2_{bbe}$ monolayers with wild-type JH642 CM, but not with Rsm121 CM, protected cells against oxidant-induced injury (monochloramine, 0.3 mM). An unrelated quorum-sensing molecule from Enterococcus (cPD1) and scrambled pentapeptide of CSF (EMTRG) had no effects. HBS, HEPES buffered saline.

(B) Silencing of OCTN2 with siRNA in Caco2 cells inhibits the protective effects of CSF (third set from left) against oxidant-induced stress, as measured by ^{51}Cr release. Clear bars indicate control cells (no CSF treatment), whereas solid bars indicates CSF-treated cells. Cells treated with a nonsense siRNA still showed protection with CSF (second set from left), indicating specificity of siRNA treatment. In the right panel, inhibitors of the Akt (Ly294002) and p38 MAPK (SB203580) pathways, either alone or together, did not significantly inhibit the CSF protective action against oxidant stress.

(C) When these agents were tested to determine their ability to limit oxidant (monochloramine)-induced decreases in barrier function, only CSF and CM of *B. subtilis* mitigated induced increases in ^{3}H-mannitol flux, a measure of barrier function. $^{2+}p < 0.05$ by ANOVA compared with untreated HBS^{+} control (n = 5 for all).

(D and E) Silencing of Hsp27 resulted in nearly complete reversal of the CSF- and BS-CM-induced protection of cell viability (Figure 3C) and epithelial barrier function (Figure 3D) against oxidant-induced stress. siRNA to murine Hsp25 (mHsp25) having nucleotide sequence unrelated to hHsp27 had no effects, indicating the specificity of Hsp27 silencing. Data are means ± SEM for the number of experiments indicated. $^{*}p \leq 0.01$ compared with corresponding responses in absence of CSF or BS-CM, n = 4.

from Rsm121 (delta – ΔCSF) did not (Figure 3A, left panel). When cells were treated with CSF, cPD1, an unrelated quorum-sensing peptide from Enterococcus (Figure 3A, middle panel), or scrambled pentapeptide of CSF (EMTRG) (Figure 3A, right panel) (all used at 100 nM, a concentration of CSF which maximally induced Hsp27 and activated p38 MAP kinase and Akt), protection against oxidant stress was only seen with CSF. Silencing of OCTN2 with siRNA inhibited the protective effects of CSF against oxidant-induced stress in $Caco2_{bbe}$ cells (Figure 3B).

Because intestinal barrier function and viability are highly sensitive to inflammation-associated injury, the above agents were tested to determine their ability to limit oxidant (monochloramine)-induced decreases in barrier function. Only CSF and CM of *B. subtilis* induced increases in ^{3}H-mannitol flux, a measure of barrier function (Figure 3C). Silencing of Hsp27 resulted in nearly complete reversal of the CSF- and CM-induced protection of cell viability (Figure 3D) and epithelial barrier function (Figure 3E) against oxidant-induced stress. In contrast, treatment of human $Caco2_{bbe}$ cells with siRNA to murine

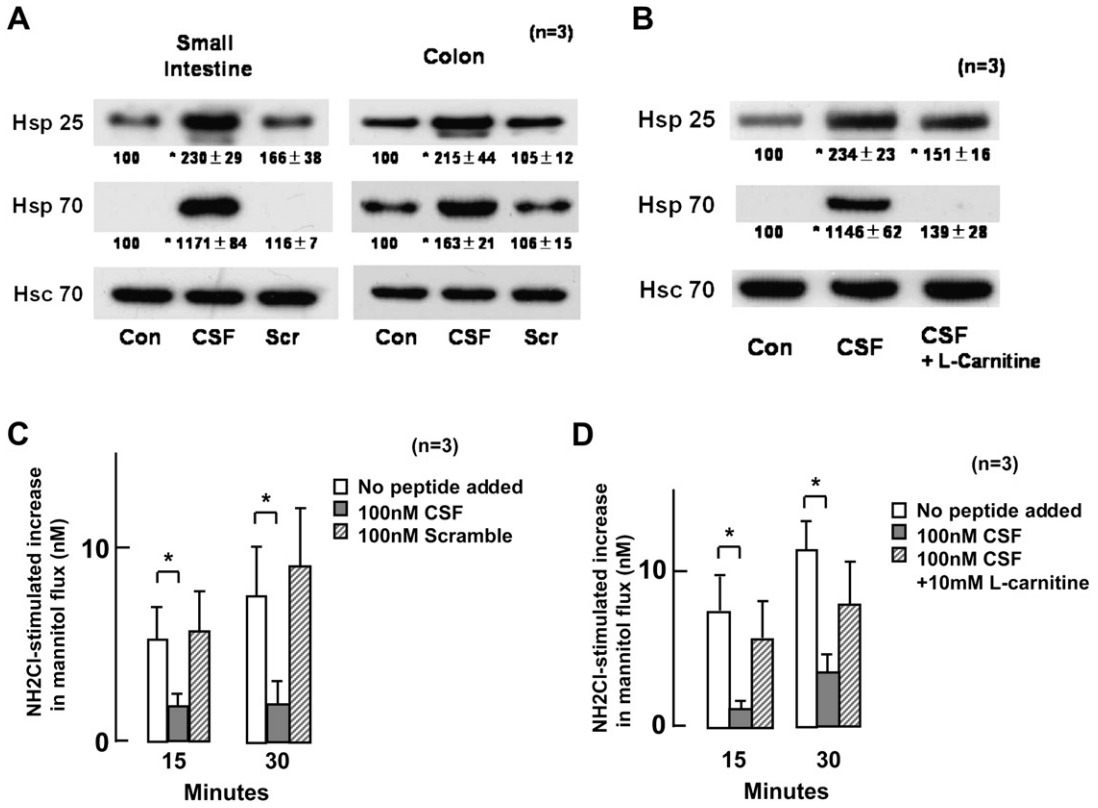

Figure 4. CSF Induces Hsp25 and Hsp70 Expression and Inhibits Oxidant-Induced Alterations in Ex Vivo Intestinal Preparations, Effects that Are Blocked by the Presence of L-carnitine

(A) By western blot, CSF (100 nM), but not the scrambled peptide (EMTRG), induces both Hsp25 and Hsp70 in mucosa of ex vivo ligated loops of murine small and large intestine (colon, left).

(B) By western blot, the induction of Hsp25 and Hsp70 by CSF is inhibited by L-carnitine in the small intestine (10 mM, right). "Con" and "Scr" indicate tissues that were not treated and were treated with the scrambled pentapeptide EMTRG, respectively.

(C) CSF, but not the scrambled peptide protects intestinal barrier function against oxidant-induced stress (NH_2Cl, 0.3 mM).

(D) CSF protection of oxidant-induced loss of barrier function (indicated by high mannitol flux) is reversed in the presence of L-carnitine, indicating an effect requiring OCTN2 transport of CSF. No treatment (clear bars) indicates oxidant effects in absence of either CSF or scrambled peptide. Permeability was assessed by passive ^{3}H-mannitol flux, calculated by subtracting the value of NH_2Cl-free from that of NH_2Cl-treated samples. Data are means ± SEM for the number of experiments indicated. *$p \leq 0.01$ compared with no treatment, n = 3.

Hsp25 (mHsp25) had no effects, indicating the specificity of Hsp27 silencing.

CSF Protects Intestinal Tissues from Oxidant Stress through OCTN2 Transport in Ex Vivo Preparation of Mice

We next examined the effects of CSF (ERGMT) and a scrambled pentapeptide molecule (EMTRG) on induction of Hsp25 and Hsp70 in ex vivo preparations of murine proximal small intestine and colon. Surgically removed segments of bowel were ligated at both ends, filled with buffer-containing CSF or scrambled peptide. After 2 hr, CSF treatment of both small and large intestinal mucosa stimulated a significant increase in Hsp25 and Hsp70 (Figure 4A), best appreciated in small intestine, as basal levels of these proteins were minimal. In contrast, no changes in mucosal Hsc70 (constitutively expressed heat shock cognate) were noted. Despite the higher basal expression in colonic mucosa due to the presence of enteric flora (Arvans et al., 2004), significant increases in Hsp25 and Hsp70 expression were still observed. In contrast, the scrambled peptide EMTRG slightly induced Hsp25, but not Hsp70, in small intestine. Thus, we cannot rule out the possibility that pentapeptides with similar amino-acid sequences have biological activity in eukaryotic cells. No induction of Hsp25 and Hsp70 was noted in large bowel mucosa exposed to the scrambled peptide. This effect was mediated by OCTN2, as L-carnitine (10 mM) inhibited CSF-induced Hsp25 and Hsp70 induction (Figure 4B). To determine the physiological consequences of the CSF effect, transmural ^{3}H-mannitol fluxes were performed in intact small bowel loops to assess intestinal barrier function. As shown in Figure 4C, increased mucosal permeability in small intestinal loops caused by exposure to oxidant (NH_2Cl 0.3 mM) was significantly inhibited by luminal CSF (100 nM), but not scrambled peptide. This protective action was inhibited when studies were performed with L-carnitine (10 mM), used to competitively

inhibit CSF uptake through OCTN2 (Figure 4D). No changes in the basal mannitol permeability were observed with either CSF or the scrambled peptide (data not shown). Additionally, pretreatment of intestinal loops or $Caco2_{bbe}$ monolayers with inhibitors of the Akt and p38 MAPK pathways (LY294002 and SB203580, respectively) had no significant effects on CSF protection (Figure S5), suggesting the induction of heat shock proteins plays a major role in conferring protection in this form of stress. While a contributory role of the Akt and p38 MAPK pathways cannot be categorically ruled out, several other reports have also demonstrated induced heat shock proteins are particularly effective in protecting cells against oxidant-induced stress (Ropeleski et al., 2003 and Arrigo et al., 2005).

DISCUSSION

This study establishes a physiological role for OCTN2-transport of CSF as a mediator of host-microbial interaction. It is notable that polymorphisms of the OCTN1 and OCTN2 encoding genes, SLC22A4 and SLC22A5, are within the IBD5 susceptibility locus of Crohn's disease (Peltekova et al., 2004; Noble et al., 2005; Vermeire et al., 2005; Walters and Sperandio, 2006; Leung et al., 2006; Waller et al., 2006), although a disease-causing role for these genes has not been established (Trinh and Rioux, 2005). OCTN2 and other similar pathways for engaging or uptake of QSMs may be essential for the regulation of host responses important for maintenance of intestinal homeostasis. Bacteria use quorum sensing to communicate and coordinate population behavior in response to environmental changes, nutrient availability, and resisting other competing or pathogenic microorganisms (Bassler and Losick, 2006 and Camilli and Bassler, 2006). Similarly, pathogenic bacteria use quorum sensing to coordinate their virulence, allowing them to evade immune detection and successfully establish infection. The finding that many Gram-positive bacteria, in contrast to Gram-negative organisms, produce compounds that compete L-carnitine uptake (Figure 2D) is interesting because their quorum-sensing molecules are typically small peptides, whereas the latter utilize nonpeptides (Bassler and Losick, 2006; Camilli and Bassler, 2006; Kendall and Sperandio, 2007). It is notable that many gut bacterial species are known to have quorum-sensing systems (Bassler and Losick, 2006; Walters and Sperandio, 2006; Kendall and Sperandio, 2007). At any point in time, the profile of quorum-signaling molecules potentially serves as a composite measure of the status of the colonic microbiota. While the study of QSMs in the colon is still in its infancy, we believe that these molecules play a major role in behavior modification and niche development of bacterial species in the gut (Kendall and Sperandio, 2007). Scott et al. (2006), for instance, reported a whole genome transcription profiling of the human gut bacterium *Roseburia inulinvorans* when grown in fucose, a human-derived sugar. This organism and related species are anaerobic polysaccharide-utilizing Firmicute bacteria that can comprise up to 10% of the total bacterial population in human feces (Hold et al., 2003). When grown with fucose, one of the major genes upregulated was a Gram-positive, *agr*-type, quorum-sensing system. The authors speculated that bacterial QSMs were likely to play a role in bacterial colonization of mucins because biofilm formation at a critical cell density would be required. Although a specific mediator was not identified, Hooper, et al. (1999) showed that *B. thetaiotamicron*, a major component of colonic flora, was able to signal to the host to produce more fucosylated glycoproteins. Sperandio, et al. (2003) also showed that the microbial intestinal flora from anaerobically cultured stools from healthy human volunteers produced two autoinducers, AI-2 and AI-3. Finally, Casula and Cutting (2002) demonstrated that orally administered *B. subtilis* germinated spores in the murine gastrointestinal tract, an action that might require initiation by QSMs like CSF.

Thus, the uptake or sampling of QSMs by OCTN2 and potentially other transporters like it may provide the host with the ability to respond or adapt to changes in the microbiome in order to maintain intestinal homeostasis. Furthermore, our in vitro study with OCTN2 siRNA strongly suggested the relevance of OCTN2 transport in inducing cytoprotective protein Hsps and protecting intestinal epithelial cells by CSF. In this regard, OCTN2 could potentially mediate some the actions of probiotic microorganisms. From our studies, it is difficult to determine the relative contribution of OCTN2-mediated host-microbial interaction relative to other forms of host-microbe interaction, including pattern recognition receptors or cytoplasmic nucleotide-binding oligomerization domain (NOD) molecules (Mueller and Podolsky, 2005). This issue will only be resolved by analysis in animals bearing intestinal, epithelial-specific, gene-targeted deletion of OCTN2. However, differences in the OCTN2 pathway from TLR and NOD signaling bear further discussion. The latter receptors are critically important for recognition of microbial-derived cellular or cell wall-derived ligands that are indicative of potential or impending threats by pathogens. As a consequence, innate immune cells can respond rapidly and appropriately to many types of pathogens. In contrast, OCTN2 is primarily expressed by intestinal epithelial cells and, to a far lesser extent, by innate immune cells. Thus, OCTN2 is less likely to be involved in meeting pathogen threats head on. While being a fairly promiscuous transporter capable of taking up many molecules, OCTN2 still requires certain structural features (e.g., small organic cations) that is likely to restrict substrates to particular types or classes of bacterial-derived molecules. Our studies would suggest that small peptide quorum-sensing molecules secreted primarily by Gram-positive bacteria are among these molecules. It is also notable that many of these bacteria are not pathogenic in the normal host and, in some cases, have been used as probiotic agents. Instead, we propose that OCTN2 is an example of a host mechanism that continuously samples the luminal content for certain microbial constituents within the enteric microbiome, allowing the host to adjust

to perturbations or changes that might otherwise affect intestinal homeostasis. Since OCTN2 function is restricted to particular substrates, we predict that other, similar pathways exist (e.g., OCTN1, MDR-1), allowing the host to survey many constituents of the microbiome. At the moment, the intracellular mammalian receptors or targets for bacterial QSM have not been identified. However, regardless of the nature of these intracellular QSM-receptors, we have demonstrated that the ability of epithelial cells to sense bacterial QSM and deliver them using highly specialized transporter molecule OCTN2. Once taken up by intestinal epithelial cells, CSF activates key survival pathways including p38 MAP kinase and protein kinase B (Akt) and induces cytoprotective heat shock proteins, the latter preventing oxidant-induced intestinal epithelial cell injury and loss of barrier function.

EXPERIMENTAL PROCEDURES

Materials

Peptides (CSF and all analogs and cPD1) were purchased from EZ Biolab (Westfield, IN) or Elim Biopharmaceuticals (Hayward, CA) and [^{3}H]-carnitine, [^{14}C]-acetic anhydride, [^{3}H]-mannitol, [^{35}S]-EXPRESS, and [^{51}Cr] Cl from Perkin Elmer (Boston, MA). Radiolabeled CSF was made by an exchange method using [1-^{14}C] acetic anhydride (Moravek Biochemicals, Brea CA). Briefly, 1.9 mg of peptide (CSF [ERGMT]) was dissolved in 100 μl water and mixed with 1 ml of sodium acetate-saturated water. Two μl of acetic anhydride were added five times at 15 min intervals at 0°C, and the reaction stopped by addition of 50 μl ammonium hydroxide. The acetylated peptide was purified by HPLC using a 5 μ 25 cm length 4.6 mm diameter Lichrosphere-100 RP-18 using a solvent gradient: starting at 98% A/2% B (A = 0.1% TFA in water and B = 0.1% TFA in acetonitrile) for 5 min and then a linear gradient to 75% A 25% B over 20 min at flow rate of 1 ml/min. Detection was at 220 nm. The fraction containing acetylated ERGMT was analyzed by nuclear magnetic resonance (NMR) spectrometry to confirm structure and identify the acetylated residues (determined to be on amino terminal E).

Cell Culture

Human colonic epithelial Caco2$_{bbe}$ cells, a generous gift from Dr. Mark Mooseker (Yale University, New Haven, CT), were grown in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum (FBS), 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 10 μg/ml transferrin (all from Invitrogen/GIBCO, Grand Island, NY) in a humidified atmosphere of 5% CO_2. Cells were used between passages 55–70. Cells were plated on 6- or 12-well plates at a density of 10^5 cells/cm^2 and were allowed to differentiate for 10–14 days before experiments.

Bacterial Strains and Preparation of Conditioned Media

B. subtilis JH642 and Rsm121 were a generous gift from Alan Grossman (MIT, Cambridge, MA). Some strains were purchased from ATCC: Bifidobacterium breve 15700, Enterococcus faecalis 10741, Enterobacter aerogenes 29007, and Proteus mirabilis 14273. Lactobacillus plantarum was a gift from S. Bengmark (Uppsala, Sweden), *E. coli* Nissle was a gift from Ardeytech in Germany, and *Lactobacillus GG* (*Lactobacillus rhamnosus GG*) was purchased from Culturelle (Bloomfield, CT). For production of conditioned media, strains were grown in overnight in either Lactobacillus MRS broth (*Lactobacillus* GG *and plantarum and Bifobacterium* [the only bacteria grown anaerobically which required two days of growth rather than overnight]) or LB broth for all remaining bacteria. Bacteria were then pelleted (2000 × g for 15 min) and washed three times in minimum medium (S7 minimal salts supplemented with 1% wt/vol glucose, 1% wt/vol glutamate, 2 mM glutamine, and Minimum Essential Medium [MEM] amino acids [Invitrogen]). Bacteria were then resuspended in 10 ml of minimal medium and incubated at 37°C while stationary for an additional 16–24 hr. Cells were then pelleted and the supernatant (containing secreted products) was filter-sterilized through a 0.1 μ filter and stored at −80°C until use. For radioactively labeled media, [^{35}S]-methionine and cysteine (^{35}S-EXPRESS labeling reagent, Perkin Elmer Radiochemicals, Boston, MA) were added to the LB or MRS growth medium for incorporation into the bacteria.

Mice

The studies were approved by the Institutional Animal Care and Use Committee of the University of Chicago. C57Bl/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) or Taconic Labs (Germantown, NY). Small and large intestines with or without treatments were removed, rinsed with ice-cold saline, and epithelium was gently sheared off with glass slides for protein or mRNA determination.

OCTN2 cDNA Transfection

Human OCTN2 (SLC22A5) cDNA (a generous gift from Dr. Vadivel Ganapathy, Medical College of Georgia, Augusta, Georgia) was sublcloned into pDsRED2-C1 (Takara/Clontech, Palo Alto, CA), and transfected Caco2$_{bbe}$ and HSWP cells using the polyamine-derived reagent LT-1 (Mirus, Madison, WI). Clones were selected on the basis of G418 (600 μg/ml) resistance and individually propagated for flux studies. The degree of OCTN2 transfection of cells was assessed by measuring Na^+-dependent L-carnitine uptake and OCTN2 immunoblotting.

siRNA

To specifically inhibit expression of OCTN2, the Invitrogen BLOCK-iT RNAi designer (Invitrogen, Carlsbad, CA) was used to select the region of the coding sequence of human OCTN2 (1331–1355) for silencing and non-sense sequence (5′-CCATCTAAGTTGCCCGTGAATCGTT-3′) as a negative control. dsRNA Stealth oligo was mixed with siLentfect reagent (Bio-Rad, Hercules, CA; 0.6 μl of reagent per cm^2 growing surface) in Optimem medium (Invitrogen) and allow to form complexes for 15 min. Sufficient dsRNA was used for a final concentration of 100 nM. Complexes were applied when cells were 60% confluent and added for a second time after 2 days. Uptake studies were performed 24–48 hr after second application.

Western Blotting

Proteins of Caco2$_{bbe}$ cells or mouse intestinal epithelia were analyzed by western blotting. Twenty to forty μg of each sample was resolved by SDS-PAGE (10%–12%) and immediately transferred to a polyvinylidene difluoride (PVDF) membrane using 1× Towbin buffer (25 mM Tris pH 8.8, 192 mM glycine with 15% [vol/vol] methanol). PVDF membranes were incubated in TBS with 0.05% (vol/vol) Tween 20 (T-TBS) containing 3% (wt/vol) BSA for 1 hr at room temperature to block nonspecific binding. Blots were incubated overnight at 4°C with the following primary antibodies: anti-mouse Hsp25 antibody (Stressgen, Victoria, British Columbia, Canada), anti-human Hsp27 (Stressgen) or anti-mouse Hsp70 antibodies (Stressgen), anti-total and phosphorylated antibodies to each of the following Akt, p38 MAP kinase, ERK 1/2 (p44/42), SAPK/JNK (Cell Signaling, Beverly, MA), rabbit polyclonal OCTN2 antiserum (Alpha Diagnostic International, San Antonio, TX), and rabbit polyclonal anti-human PepT1 (a gift from Didier Merlin, Emory University). PepT1 (D. Merlin, et al.) Blots were washed five times for 10 min each in T-TBS at room temperature, incubated for 60 min in species-appropriate horseradish peroxidase-conjugated secondary antibody (Jackson Immunoresearch, West Grove, PA) T-TBS, washed four times in T-TBS, once in TBS, and developed using the Super-Signal West Pico enhanced chemiluminescence system (Pierce Chemical, Rockford, IL).

Cell Viability Assay

Caco2$_{bbe}$ cells were grown in 24-well plates until differentiated and then treated with 10% vol/vol of *B. subtilis*-conditioned medium,

100 nM CSF or other peptides for 24 hr. Cells were loaded with ^{51}Cr (50 μCi/ml) for 60 min, washed, and incubated in media with 0.6 mM monochloramine to induce cell injury. Medium was harvested from the cells after 60 min, and the ^{51}Cr remaining in the cells was extracted with 0.1 wt/vol% SDS. The amounts of ^{51}Cr in the released and cellular fractions were counted by liquid scintillation spectrometer. The amount of ^{51}Cr released was calculated as the amount released divided by the total (cellular and released) ^{51}Cr.

Ex Vivo Intestinal Loop Studies

C57Bl/6 mice (18–25 gm) were sacrificed and the small intestine was removed beginning at the ligament of Treitz. The first 18 cm were divided into three 6 cm lengths, each end ligated with silk suture and the loops filled with RPMI 1640 medium with 10% vol/vol heat inactivated FBS, with or without peptides (ERGMT [CSF] or EMTRG [scrambled]) at 100 nM. Loops were filled to moderate distention, about 1 ml per loop. Loops were placed in the outer loop of organ culture dishes (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) which were filled with 5 ml media as above. Loops were incubated for 2 hr at 37°C in a 5% CO_2 incubator. A 1 cm segment was removed from the middle and mucosa scraped off with glass slides and processed for protein analysis as described in detail in the supplement. To measure permeability effects, the two remaining segments were filled with RPMI 1640 medium containing serum with 1 mM mannitol and 1 μCi/ml [^{3}H]-mannitol, and with or without 0.3 mM freshly prepared monochloramine. Loops were placed into the middle section of the organ culture dish in 2 ml of RPMI 1640 with serum without NH_2Cl. Samples were taken at 5, 20, and 35 min to determine flux of mannitol from lumen to medium outside bathing loops.

Supplemental Data

The Supplemental Data include Supplemental Experimental Procedures and five supplemental figures and can be found with this article online at http://www.cellhostandmicrobe.com/cgi/content/full/1/4/299/DC1/.

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REFERENCES

Alexopoulos, C., Georgoulakis, I.E., Tzivara, A., Kyriakis, C.S., Govaris, A., and Kyriakis, S.C. (2004). Field evaluation of the effect of a probiotic-containing Bacillus licheniformis and Bacillus subtilis spores on the health status, performance, and carcass quality of grower and finisher pigs. J. Vet. Med. A Physiol. Pathol. Clin. Med. *51*, 306–312.

Arrigo, A.P., Virot, S., Chaufour, S., Firdaus, W., Kretz-Remy, C., and Diaz-Latoud, C. (2005). Hsp27 consolidates intracellular redox homeostasis by upholding glutathione in its reduced form and by decreasing iron intracellular levels. Antioxid. Redox Signal. *7*, 414–422.

Arvans, D.L., Vavricka, S.R., Ren, H., Musch, M.W., Kang, L., Rocha, F.G., Lucioni, A., Turner, J.R., Alverdy, J., and Chang, E.B. (2004). Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. Am. J. Physiol. Gastrointest. Liver Physiol. *288*, G696–G704.

Barbosa, T.M., Serra, C.R., La Ragione, R.M., Woodward, M.J., and Henriques, A.O. (2005). Screening for bacillus isolates in the broiler gastrointestinal tract. Appl. Environ. Microbiol. *71*, 968–978.

Bassler, B.L., and Losick, R. (2006). Bacterially speaking. Cell *125*, 237–246.

Benno, Y., Endo, K., Mizutani, T., Namba, Y., Komori, T., and Mitsuoka, T. (1989). Comparison of fecal microflora of elderly persons in rural and urban areas of Japan. Appl. Environ. Microbiol. *55*, 1100–1105.

Camilli, A., and Bassler, B.L. (2006). Bacterial small-molecule signaling pathways. Science *311*, 1113–1116.

Casula, G., and Cutting, S.M. (2002). Bacillus probiotics: Spore germination in the gastrointestinal tract. Appl. Environ. Microbiol. *68*, 2344–2352.

Charrier, L., Driss, A., Yan, Y., Nduati, V., Klapproth, J.M., Sitaraman, S.V., and Merlin, D. (2006). hPepT1 mediates bacterial tripeptide fMLP uptake in human monocytes. Lab. Invest. *86*, 490–503.

D'Arienzo, R., Maurano, F., Mazzarella, G., Luongo, D., Stefanile, R., Ricca, E., and Rossi, M. (2006). Bacillus subtilis spores reduce susceptibility to Citrobacter rodentium-mediated enteropathy in a mouse model. Res. Microbiol. *157*, 891–897.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora. Science *308*, 1635–1638.

Hold, G.L., Schwiertz, A., Aminov, R.I., Blaut, M., and Flint, H.J. (2003). Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. Appl. Environ. Microbiol. *69*, 4320–4324.

Hooper, L.V., Xu, J., Falk, P.G., Midtvedt, T., and Gordon, J.I. (1999). A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc. Natl. Acad. Sci. USA *96*, 9833–9838.

Hooper, L.V., Wong, M.H., Thelin, A., Hansson, L., Falk, P.G., and Gordon, J.I. (2001). Molecular analysis of commensal host-microbial relationships in the intestine. Science *291*, 881–884.

Kendall, M.M., and Sperandio, V. (2007). Quorum sensing by enteric pathogens. Curr. Opin. Gastroenterol. *23*, 10–15.

Kravchenko, V.V., Kaufmann, G.F., Mathison, J.C., Scott, D.A., Katz, A.Z., Wood, M.R., Brogan, A.P., Lehmann, M., Mee, J.M., Iwata, K., et al. (2006). N-(3-oxo-acyl)homoserine lactones signal cell activation through a mechanism distinct from the canonical pathogen-associated molecular pattern recognition receptor pathways. J. Biol. Chem. *281*, 28822–28830.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P., Bolotin, A., Borchert, S., et al. (1997). The complete genome sequence of the Gram-positive bacterium Bacillus subtilis. Nature *390*, 249–256.

Lazazzera, B.A., Solomon, J.M., and Grossman, A.D. (1997). An exported peptide functions intracellularly to contribute to cell density signaling in B. subtilis. Cell *89*, 917–925.

Leung, E., Hong, J., Fraser, A.G., Merriman, T.R., Vishnu, P., and Krissansen, G.W. (2006). Polymorphisms in the organic cation transporter genes SLC22A4 and SLC22A5 and Crohn's disease in a New Zealand Caucasian cohort. Immunol. Cell Biol. *84*, 233–236.

Levin, P.A., and Grossman, A.D. (1998). Cell cycle and sporulation in Bacillus subtilis. Curr. Opin. Microbiol. *1*, 630–635.

Morimoto, R.I. (1993). Cells in stress: Transcriptional activation of heat shock genes. Science *259*, 1409–1410.

Morimoto, R.I. (2002). Dynamic remodeling of transcription complexes by molecular chaperones. Cell *110*, 281–284.

Mueller, T., and Podolsky, D.K. (2005). Nucleotide-binding-oligomerization domain proteins and toll-like receptors: Sensors of the inflammatory bowel diseases' microbial environment. Curr. Opin. Gastroenterol. *21*, 419–425.

Noble, C.L., Nimmo, E.R., Drummond, H., Ho, G.T., Tenesa, A., Smith, L., Anderson, N., Arnott, I.D., and Satsangi, J. (2005). The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. Gastroenterology *129*, 1854–1864.

Ohashi, R., Tamai, I., Nezu, Ji.J., Nikaido, H., Hashimoto, N., Oku, A., Sai, Y., Shimane, M., and Tsuji, A. (2001). Molecular and physiological evidence for multifunctionality of carnitine/organic cation transporter OCTN2. Mol. Pharmacol. *59*, 358–366.

Pasare, C., and Medzhitov, R. (2005). Toll-like receptors: Linking innate and adaptive immunity. Adv. Exp. Med. Biol. *560*, 11–18.

Peltekova, V.D., Wintle, R.F., Rubin, L.A., Amos, C.I., Huang, Q., Gu, X., Newman, B., Van Oene, M., Cescon, D., Greenberg, G., et al. (2004). Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat. Genet. *36*, 471–475.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell *118*, 229–241.

Rakoff-Nahoum, S., and Medzhitov, R. (2006). Role of the innate immune system and host-commensal mutualism. Curr. Top. Microbiol. Immunol. *308*, 1–18.

Rhee, K.J., Sethupathi, P., Driks, A., Lanning, D.K., and Knight, K.L. (2004). Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. J. Immunol. *172*, 1118–1124.

Ropeleski, M.J., Tang, J., Walsh-Reitz, M.M., Musch, M.W., Larson, R.A., and Chang, E.B. (2003). Interleukin-11-induced heat shock protein 25 confers intestinal epithelial-specific cytoprotection from oxidant stress. Gastroenterology *124*, 1358–1368.

Scott, K.P., Martin, J.C., Campbell, G., Mayer, C.D., and Flint, H.J. (2006). Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "Roseburia inulinivorans". J. Bacteriol. *188*, 4340–4349.

Solomon, J.M., Lazazzera, B.A., and Grossman, A.D. (1996). Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in Bacillus subtilis. Genes Dev. *10*, 2014–2024.

Sperandio, V., Torres, A.G., Jarvis, B., Nataro, J.P., and Kaper, J.B. (2003). Bacteria-host communication: The language of hormones. Proc. Natl. Acad. Sci. USA *100*, 8951–8956.

Tam, N.K., Uyen, N.Q., Hong, H.A., Duc le, H., Hoa, T.T., Serra, C.R., Henriques, A.O., and Cutting, S.M. (2006). The intestinal life cycle of Bacillus subtilis and close relatives. J. Bacteriol. *188*, 2692–2700.

Tamai, I., Ohashi, R., Nezu, J.I., Sai, Y., Kobayashi, D., Oku, A., Shimane, M., and Tsuji, A. (2000). Molecular and functional characterization of organic cation/carnitine transporter family in mice. J. Biol. Chem. *275*, 40064–40072.

Tao, Y., Drabik, K.A., Waypa, T.S., Musch, M.W., Alverdy, J.C., Schneewind, O., Chang, E.B., and Petrof, E.O. (2006). Soluble factors from Lactobacillus GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells. Am. J. Physiol. *290*, C1018–C1030.

Trinh, T.T., and Rioux, J.D. (2005). Understanding association and causality in the genetic studies of inflammatory bowel disease. Gastroenterology *129*, 2106–2110.

Vermeire, S., Pierik, M., Hlavaty, T., Claessens, G., van Schuerbeeck, N., Joossens, S., Ferrante, M., Henckaerts, L., Bueno de Mesquita, M., Vlietinck, R., and Rutgeerts, P. (2005). Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. Gastroenterology *129*, 1845–1853.

Vikstrom, E., Magnusson, K.E., and Pivorinas, A. (2005). The Pseudomonas aeruginosa quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone stimulates phagocytic activity in human macrophages through the p38 MAPK pathway. (2005). Microbes Infect. *7*, 1512–1518.

Vikstrom, E., Tafazoli, F., and Magnusson, K.E. (2006). Pseudomonas aeruginosa quorum sensing molecule N-(3 oxododecanoyl)-l-homoserine lactone disrupts epithelial barrier integrity of Caco-2 cells. FEBS Lett. *580*, 6921–6928.

Waller, S., Tremelling, M., Bredin, F., Godfrey, L., Howson, J., and Parkes, M. (2006). Evidence for association of OCTN genes and IBD5 with ulcerative colitis. Gut. *55*, 809–814.

Walters, M., and Sperandio, V. (2006). Quorum sensing in Escherichia coli and Salmonella. Int. J. Med. Microbiol. *296*, 125–131.

Zhang, H.M., Rao, J.N., Guo, X., Liu, L., Zou, T., Turner, D.J., and Wang, J.Y. (2004). Akt kinase activation blocks apoptosis in intestinal epithelial cells by inhibiting caspase-3 after polyamine depletion. J. Biol. Chem. *279*, 22539–22547.