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The role of the commensal microbiota in adaptive and maladaptive stressor-induced immunomodulation

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Abstract

Over the past decade, it has become increasingly evident that there are extensive bidirectional interactions between the body and its microbiota. These interactions are evident during stressful periods, where it is recognized that commensal microbiota community structure is significantly changed. Many different stressors, ranging from early life stressors to stressors administered during adulthood, lead to significant, community-wide differences in the microbiota. The mechanisms through which this occurs are not yet known, but it is known that commensal microbes can recognize, and respond to, mammalian hormones and neurotransmitters, including those that are involved with the physiological response to stressful stimuli. In addition, the physiological stress response also changes many aspects of gastrointestinal physiology that can impact microbial community composition. Thus, there are many routes through which microbial community composition might be disrupted during stressful periods. The implications of these disruptions in commensal microbial communities for host health are still not well understood, but the commensal microbiota have been linked to stressor-induced immunopotentiation. The role of the microbiota in stressor-induced immunopotentiation can be adaptive, such as when these microbes stimulate innate defenses against bacterial infection. However, the commensal microbiota can also lead to maladaptive immune responses during stressor-exposure. This is evident in animal models of colonic inflammation where stressor exposure increases the inflammation through mechanisms involving the microbiota. It is likely that during stressor exposure, immune cell functioning is regulated by combined effects of both neurotransmitters/hormones and commensal microbes. Defining this regulation should be a focus of future studies.

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Introduction

Microbes are found on every surface of the body (including cutaneous and mucosal surfaces). All types of microbes, including bacteria, archaea, protists, and viruses, can naturally colonize the body, but the vast majority of these microbes are bacteria that naturally reside within the intestines as part of the intestinal microbiota. Bacteria can be found in all sections of the gastrointestinal tract, but the levels of these microbes differ dramatically depending upon gastrointestinal section. In general, bacterial levels are low in the stomach and upper parts of the small intestine reaching only 10^2 to 10^3 bacteria per ml of intestinal contents. But, bacterial numbers are significantly higher in the distal portion of the small intestine and large intestine such that numbers of bacteria in the large intestine are as high 10^{12} bacteria per ml of intestinal contents. Approximately 90–95% of these bacteria are members of 2 bacterial phyla, the Bacteroidetes and Firmicutes, with a smaller proportion of these bacteria belong to the Actinobacteria, Proteobacteria, Deferribacteres, TM7, Deinococcus, and Fusobacteria phyla (Eckburg et al., 2005; Stearns et al., 2011). These relatively few bacterial phyla give rise to over 500 different species of bacteria, thus demonstrating the enormous bacterial diversity that is naturally found on the human body.

The microbiota form well defined communities within their niche, allowing the microbes to interact with each other and also interact with their host (Sekirov et al., 2010). Over the past few years, microbial ecologists have spent significant time and effort characterizing the structures of these microbial communities. In general, the structure of the microbial community refers the relative proportions of the different microbes within the community. There are two common types of measures that characterize the diversity of a microbial community that are commonly used to describe the structure of the microbial community(Sekirov et al., 2010). The first type involves measures of alpha diversity. Alpha diversity encompasses two measures of the microbial community, richness of species and evenness of species. Richness of species simply refers to the number of different types of bacteria within a community, whereas as evenness refers to the distribution of individual bacterial types. In addition to alpha diversity, measures of beta diversity are commonly used to characterize microbial communities. Beta diversity refers to diversity of microbes compared across different samples, and may or may not take into consideration the relative abundances of different microbes(Sekirov et al., 2010).

The factors that determine the structure of microbial communities within the intestines are not yet well defined. However, it is known that intrinsic factors, such as host genetics and immune system activity, as well as extrinsic factors, such as antibiotics and host diet, play important roles in shaping microbial community structure and function(Antonopoulos, 2009; Dethlefsen, 2008; Turnbaugh et al., 2008). To some extent, the impact that these factors can have on the microbiota are logical and very intuitive. It is less intuitive that psychosocial factors can also impact the composition of the microbiota. However, studies from this lab and others have found that exposure to stressful stimuli is sufficient to significantly change the composition of the gut microbiota.

The physiological Stress Response

Stress is an intrinsic part of life, and successfully adapting to stimuli that induce stress is necessary for the survival of an organism in its environment that is constantly changing. Stress is a process in which a stimulus (termed the stressor) disrupts internal homeostasis. The stressor can be physical (e.g., environmental temperature), physiological (e.g., altered glucose levels), or psychological (e.g., social defeat) in nature, and sets into a motion a series of physiological and behavioral responses (termed the stress response) that are meant to respond to and cope with the original stressor. Two neuroendocrine pathways that are commonly activated during a stress response are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). Activation of the HPA axis results in increased production of adrenocorticotrophin (ACTH) by the pituitary gland. The ACTH enters into circulation where it stimulates the adrenal cortex to produce mineralcorticoid and glucocorticoid hormones. Activation of the HPA axis occurs over a period of several minutes, in contrast to activation of the SNS which becomes activated in a matter of seconds. Activation of the SNS results in the release of norepinephrine from the nerve terminals in SNS-innervated tissues. Most organs are innervated by the SNS and receptors for the catecholamines and glucocorticoid hormones are widespread throughout the body. Thus, it is not surprising that these hormones have multiple effects throughout the body, such as mobilizing energy for the well-known “Fight or Flight” response. These physiological effects of SNS and HPA axis activation are all aimed at helping the body respond to the demands being placed on it and ultimately to maintain a state of internal homeostasis. However, the physiological response to stress also significantly impacts the structure and function of commensal microbes within the intestines.

Microbial Endocrinology

In the early 1990’s, Dr. Mark Lyte published the first papers clearly demonstrating that bacteria can recognize and respond to mammalian hormones (Lyte, 2016a, b). For example, when bacteria, such as pathogenic *Yersinia enterocolitica* or commensal *Escherichia coli*, are grown in a serum based, basal medium, little bacterial growth is observed over a 24–48 hour period(Freestone et al., 2002; Lyte and Ernst, 1992). However, if the exact same media is simply supplemented with norepinephrine or epinephrine, significant increases in bacterial growth are observed (Freestone et al., 2002; Lyte and Ernst, 1992). These novel findings are now highly reproducible, and some of the mechanisms by which the catecholamine group of neurotransmitters and hormones impact bacterial growth have been described(Hughes et al., 2009; Moreira et al., 2016; Moreira and Sperandio, 2012; Pullinger, 2010; Sandrini et al., 2010). Perhaps more importantly, these novel findings have led to the development of an area of research referred to as Microbial Endocrinology(Lyte, 1993, 2004, 2010, 2016a, b).

Growth is not the only bacterial function that can be impacted by the catecholamines. This is well documented for the pathogen Enterohemmorhagic *E. coli* (EHEC), which is a food-borne pathogen that can lead to hemorrhagic colitis, particularly in infants and young children as well as the elderly. Giving norepinephrine (or dopamine) to EHEC not only increases its growth, but significantly increases the ability of the microbe to adhere to the intestinal epithelium (Bansal et al., 2007; Vlisidou et al., 2004). This is important, because adherence to the intestinal tissue is an important first step in the ability of EHEC to induce

hemorrhagic colitis. After adhering to the intestinal tissue, EHEC produces a toxin, referred to as a Shiga-like toxin. Of importance, EHEC-produced Shiga-like toxin production was found to be significantly enhanced upon exposure to norepinephrine (Lyte et al., 1997).

Although most studies within the field of Microbial Endocrinology have focused on pathogenic microbes, it is also clear that commensal microbes can be affected by neuroendocrine hormones. Studies utilizing a neurotoxic drug, namely 6-hydroxydopamine (6-OHDA) have shown that commensal bacterial levels in the intestines can be impacted by chemical sympathectomy. Traditionally, 6-OHDA is used to deplete norepinephrine *in vivo* through the selective lysis of adrenergic nerve terminals. Depletion occurs over the course of approximately a week, but at earlier time points, norepinephrine levels are significantly elevated due to the release of neurotransmitters that are stored within synaptic vesicles. Studies have shown that as early as 24 hrs after treatment with 6-OHDA, a time when norepinephrine levels are significantly elevated, the levels of commensal bacteria that can be cultured from the intestines are increased approximately 1,000 – 100,000 fold(Lyte and Bailey, 1997). Interestingly, the number of bacteria associated with the intestinal tissue, as well as those in the lumen of the intestines, was also significantly increased(Lyte and Bailey, 1997). When considered with studies from *in vitro* assays involving pathogenic microbes, it is likely that the large bolus release of norepinephrine *in vivo* significantly increased bacterial growth and adherence to intestinal tissue. Such neuroendocrine-bacterial interactions could help to explain why intestinal bacteria are able to translocate from the lumen of the intestines to the interior of the body of mice exposed to stressful stimuli (Bailey et al., 2006), a hypothesis warranting further investigation.

Stress and GI Physiology

Studies in Microbial Endocrinology were highly influential on earlier studies from this laboratory assessing the microbiota in animals exposed to stressful stimuli. One hypothesis is that direct neuroendocrine-bacterial interactions can impact the composition of the gut microbiota during stressful periods (Freestone and Lyte, 2010; Lyte, 2014; Lyte et al., 2011). However, direct interaction with mammalian hormones is not the only way in which the composition of the microbiota might be impacted during stressor exposure, and it is possible that stressor-induced changes to gastrointestinal functioning may also impact microbial composition in the gut.

It has long been thought that differences in bacterial colonization along the length of the gastrointestinal tract are due to differences in normal physiological functioning and the resultant differences in the microenvironment. For example, the stomach harbors low levels of commensal microbes due to the secretion of gastric acid and the resultant high acidity; an inability to secrete gastric acid, such that occurs with achlorhydria, can result in significant increases in stomach colonization(Drasar, 1969). Similarly, motility in upper sections of the small intestine (i.e., duodenum and jejunum) is more rapid than in lower parts of the small intestine (i.e., the ileum). Thus, bacterial colonization is sparse in the duodenum and jejunum compared to the ileum(Berg, 1996).

Bacterial levels are greatest in the large intestine, where motility and the oxidation-reduction potential is the lowest(Berg, 1996). As a result, approximately 10^{10}–10^{12} of obligate

anaerobic and facultatively anaerobic bacteria can be found in a gram of colonic contents. Many of these microbes are truly in the lumen of the colon, however, some of these bacteria can also be found in the mucous layer covering the intestines. Fewer of these bacteria will actually be found adhered to intestinal epithelial cells. Of importance to this review, we have found that stressor exposure differentially effects those microbial populations found in the colonic lumen and those that are associated with the colonic mucosa (Galley et al., 2014b). Although it is not yet understood, it is possible that these mucosa-associated populations have more exposure to host-derived hormones or are more susceptible to differences in gastrointestinal physiology.

Factors secreted into the intestines during digestion or as part of the protective barrier, can also shape the intestinal microbiota. For example, bile, which is produced in the liver, but stored in the gallbladder, and released into the intestines after a meal can have multiple effects on intestinal bacteria. Bile, which is a strong detergent, can have a direct effect on bacteria by destroying bacterial membranes, or can have a more indirect effect by stimulating mucosal defenses (Inagaki et al., 2006). The importance of bile in shaping gut microbial communities was demonstrated by studies showing that biliary obstruction, which blocks the bile flow into the intestines, leads to bacterial overgrowth and translocation in the small intestine (Clements et al., 1996). Interestingly, effects on the microbiota could be reversed by administering bile acids, indicating the effects are in fact due to reduced bile acid release (Lorenzo-Zuniga et al., 2003).

Additional secretory factors, such as antimicrobial peptides (AMPs) and secretory immunoglobulin A (sIgA) can also impact microbial community composition. The AMPs, which include defensins, cathelicidins, and C-type lectins, are broad spectrum antibiotics that are largely produced by epithelial cells but can also be made by circulating inflammatory cells (Ostaff et al., 2013). Initially described by their ability to destroy ingested pathogens it has become widely accepted that their ability to shape and control the intestinal commensal microbiota is of the utmost importance for achieving intestinal homeostasis. For example, a study by the Salzman group demonstrated that Paneth cell-derived α-defensins can significantly change the overall microbial composition of the small intestine, however overall bacterial load was unchanged (Salzman, 2010). A C-type lectin, RegIIIγ, is able to control Gram positive bacterial mucous penetration thereby preventing bacterial adherence to the intestinal mucosa (Hooper et al., 2012). Together with AMPs, sIgA functions as a neutralizing agent against toxins and bacteria through various mechanisms including immune exclusion, receptor blockade and steric hindrance and in fact upwards of 70% of the intestinal commensal microbiota are coated with sIgA (Mantis, 2011; Mantis et al., 2011). The use of an IgA-deficient mouse model highlighted the importance of sIgA in controlling the overall numbers of intestinal bacteria as there were significant increases in non-pathogenic commensal bacteria and anaerobic bacteria in the small intestine (Fagarasan et al., 2002; Suzuki et al., 2004). Taken together these studies demonstrate that AMPs are heavily involved in shaping the intestinal community structure while sIgA is beneficial for controlling overall bacterial load within the intestines.

The physiological response to stress is well recognized to influence gastrointestinal physiology. In fact, the functioning of all segments of the gastrointestinal tract are changed

by stress-responsive hormones and neurotransmitter systems. For example, gastric acid secretion has been shown to be suppressed by sympathetic nervous system activation and enhanced by parasympathetic nervous system activation (Yang, 2000). Activation of the sympathetic nervous system also prevents the release of bile into the intestines from the gallbladder (Lenz, 1993; Lenz et al., 1992; Lenz et al., 1993; Messmer et al., 1993). The digestive functions of the pancreas, including the pancreatic volume, protein levels, and bicarbonate secretion, are also inhibited by activation of the sympathetic nervous system (Lenz et al., 1992). Small intestinal motility (McRae et al., 1982; Stanghellini et al., 1983, 1984; Zhao et al., 2014) and water absorption (Lenz et al., 1992) are also reduced by the sympathetic nervous system, whereas colonic motility is increased (Tache, 2001, 2004). In addition to these digestive functions, barrier functions in the intestines are also affected by stressor exposure/neuroendocrine hormones. For example, stressor exposure has been shown to affect sIgA levels in the intestines, but these effects appear to be complex and dependent upon the intensity and duration of the stressor, as well as the species/strain of laboratory animal. For example, short lasting (3 hr) restraint of Wistar rats on 7 consecutive days increased sIgA in the small intestine (Reyna-Garfias et al., 2010), whereas 4 hrs of restraint of Balb/C mice on 4 consecutive days decreased sIgA (Jarillo-Luna et al., 2007). These discrepencies are in part due to the complex interactions of hormones (such as stress-induced glucocorticoids) on B cell trafficking and differentiation, sIgA dimer formation, and secretion into the intestinal lumen (Campos-Rodriguez et al., 2013).

In addition to sIgA, stressor exposure can impact mucous levels, as well as levels of antimicrobial peptides in the intestines. For example, chronic subordinate colony housing, water avoidance stress, and water immersion, in rats significantly reduces the number of mucous-producing cells, thickness of the mucous barrier, and the production of the mucin Muc2 in the colon (Da Silva et al., 2014; Nyuyki et al., 2012; Shigeshiro, 2012). Thus, although it has been recognized for approximately 100 years that stress can change gastrointestinal system functioning, the extent of the effects of stressor exposure on GI functioning, as well as some of the mechanisms by which they occur, are increasingly well defined.

Stress and the GI Microbiota

Knowing that exposure to stressful stimuli impacts normal gastrointestinal functioning, and that microbes can be affected by gastrointestinal physiological properties, as well as hormones themselves, led us to test whether exposure to stressful situations impacted the commensal microbiota. In early studies it was found that the stress of maternal separation was sufficient to significantly reduce the number of lactobacilli that could be cultured from the intestines of 6 mo old rhesus monkeys (Bailey and Coe, 1999). Interestingly, this reduction in lactobacilli correlated with stress-indicative behaviors; animals with the strongest behavioral response to the stressor were also the animals that had the strongest reduction in lactobacilli (Bailey and Coe, 1999).

Maternal separation is not the only stressor to lead to reductions in lactobacilli. Monkeys exposed to an acoustical startle stressor during gestation were found to have lower levels of lactobacilli during the first 6 months of life (Bailey et al., 2004). Stressor-induced reductions

in the lactobacilli are not limited to rhesus monkeys. In fact, it was recognized over 40 years ago that depriving mice of bedding, as well as food and water, was sufficient to reduce the number of lactobacilli cultured in the intestines (Tannock and Savage, 1974). This result was confirmed more recently when it was found that mice housed in cages lacking bedding, as well as mice exposed to horizontal shaking, had differences in microbial community composition (Bangsgaard Bendtsen et al., 2012; Sakuma, 2013), including lower levels of lactobacilli than non-stressed control mice (Sakuma, 2013). This finding led the authors to suggest that reduction in the lactobacilli could be used as a marker for environmental stressor exposure (Sakuma, 2013).

We have often found low levels of lactobacilli in stressor exposed mice. For example, mice exposed to a prolonged restraint stressor repeated on 6 consecutive days have been shown to have reduced levels of lactobacilli in the colon (Galley et al., 2014b). Similarly, mice exposed to a model of repeated social defeat, called social disruption (SDR) that is repeated on 6 consecutive nights, have been found to have lower levels of lactobacilli that are associated with the colonic mucosa (Galley et al., 2014a). Interestingly, as little as 2 hrs of exposure to the SDR stressor is sufficient to reduce the levels of colonic mucosa-associated lactobacilli indicating that changes to the lactobacilli are initiated rapidly and persist throughout the duration of stressor exposure (Galley et al., 2014a). It is not yet known if or when lactobacilli levels return to homeostatic levels.

Although the lactobacilli are often found to be affected by stressor exposure, non-targeted assessment of the gut microbial community demonstrates that stressor exposure has a broad effect on the overall structure of the microbial communities as assessed by beta-diversity measures. For example, the use of next-generation, high throughput 454 pyrosequencing to characterize microbial community composition demonstrated that prolonged restraint significantly affected by alpha- and beta-diversity measures (Bailey et al., 2010; Galley et al., 2014b). For example, alpha diversity, which assesses diversity of species within samples, was significantly decreased in the cecum after repeated days of prolonged restraint (Bailey et al., 2010). Similarly, community beta diversity, which assesses diversity between samples, was significantly different after exposure to repeated cycles of prolonged restraint (Bailey et al., 2010). These phenomena, which were originally observed in the contents of the cecum, were also observed in microbial communities found in the lumen of the colon (i.e., in the feces prior to expulsion from the mice) and in populations associated with the colonic tissue (i.e., the mucosa-associated populations). Of importance, stressor exposure had a stronger effect on mucosa-associated populations compared to luminal/fecal populations (Galley et al., 2014b).

Our findings of stressor-induced changes in overall microbial community structure is consistent with findings from other groups. For example, early life stress (induced through maternal separation) in both rats and mice leads to significant community-wide differences in the gut microbiota (De Palma et al., 2015; O'Mahoney, 2009; Pusceddu et al., 2015). These differences in the microbiota have been associated with behavioral abnormalities when the maternally separated offspring reach adulthood. Taxonomic differences in the rat microbiota, and in behavior, could be prevented through the administration of N-3 poly-unsaturated fatty acids (PUFA), suggesting that the microbiota directly contribute to

differences in behavior (Pusceddu et al., 2015). However, in mice, transplantation of the microbiota from maternally separated mice into non-stressed germ-free recipient mice did not change behavior. However, transplanting the microbiota into maternally separated germ-free recipient mice resulted in changes in behavior (De Palma et al., 2015). This illustrates that early life stressor exposure disrupts the interactions between the host and its microbiota in biologically meaningful ways.

The effects of stress on the microbiota may actually begin prenatally. Previous studies found that exposing monkeys to stress during gestation significantly changed the numbers of lactobacilli and bifidobacteria that could be cultured from the intestines throughout the first six weeks of life (Bailey et al., 2004). Importantly, studies in mice have confirmed and extended these initial observations in rhesus monkeys. The use of culture-independent analyses indicated that stress during pregnancy significantly reduced lactobacilli levels in the maternal vagina (Jasarevic et al., 2015). Because maternal vaginal bacteria are transmitted to the offspring during birth, lactobacilli levels in the offspring were also assessed. Importantly, gut lactobacilli in mice born from dams exposed to the stressor during pregnancy were significantly lower than lactobacilli levels in mice born from non-stressed control dams. Interestingly, these changes in the microbiota were associated with changes in metabolite profiles and amino acid profiles in the developing brain, suggesting that the microbiota are key players in the impact of gestational stress on offspring neurodevelopment (Jasarevic et al., 2015). The microbiota may not only be linked to more than just neurodevelopment if altered during exposure to a prenatal stressor. A recent study indicated the effects of prenatal stress on the gut microbiota are associated with developmental differences in gastrointestinal and respiratory functioning and hyper-responsiveness of the hypothalamic-pituitary-adrenal axis to stress, showing that prenatal stress can have far-reaching effects on offspring development (Golubeva et al., 2015). Interestingly, human studies have begun to confirm that prenatal and early life stress can change the composition of the gut microbiota. In a study assessing the development of the intestinal microbiota over the first 110 days after birth in a healthy cohort of 56 vaginally born Dutch infants, investigators found that maternal prenatal stress was associated with alterations in the infant microbiota. Infants from high stress mothers had higher levels of Proteobacteria (a group of bacteria that includes many pathobionts, such as *Escherichia, Proteus*, and *Helicobacter* spp.), but lower levels of lactic acid bacteria (which includes beneficial bacteria in the genus *Lactobacillus*), and Actinobacteria (which contains the beneficial bacteria in the genus *Bifidobacterium*) (Zijlmans et al., 2015). These studies demonstrate that early life and gestational stressors change the composition of the gut microbiota in both humans and laboratory animals. Disrupted interactions between the gut microbiota and the host can have significant effects on healthy development.

Stressor-induced changes in the microbiota are not only evident with early life stressors, and several studies now support that stressor exposure in adult animals can also impact microbial community composition. In adult mice, exposure to chronic social defeat (induced by 5–10 min of social defeat followed by 24 hr of sensory contact with the aggressive conspecific) for 10 consecutive days resulted in reductions in both alpha and beta diversity measures (Bharwani et al., 2016). Similar to previous studies involving the social disruption stressor (Bailey et al., 2011), mice in the chronic social defeat paradigm showed evidence of

increased immune activation related to changes in the microbiota (Bharwani et al., 2016). In a similar study assessing microbiota, immunity, and microbial-produced metabolites, stressor-induced changes in the microbiota, cecal IgA, and cecal metabolites could be linked to differences in social behavior (Aoki-Yoshida et al., 2016). These studies consistently demonstrate that stressor exposure impacts the composition of the gut microbiota in adult animals. Thus, there is now ample evidence that exposure to stressful stimuli significantly changes the microbial community structure within the intestines. Studies have linked changes in the microbiota with host health, but fewer studies have clearly demonstrated that stressor-induced changes in microbial community composition in turn changes microbial community function.

Stress, the Microbiota, and Immunomodulation

The function of a microbial community refers to the biological activities of the microbial community. These activities can serve to benefit the community itself, or may have important effects on their host. While the structure of a microbial community is often related to the function of that community, in many cases microbial community structure-function relationships are poorly defined. However, because changes to microbial community structure can impact the production of metabolites and can impact immune system reactivity to pathogens, studies from this laboratory have sought to determine whether stressor exposure impacts the function of the microbiota by assessing the impact on systemic and mucosal immune responses. Effects of stress, and the potential role of the microbiota, on brain and behavior have been extensively reviewed elsewhere (Dinan and Cryan, 2012; Moloney et al., 2016).

There are extensive bidirectional interactions between the host and its microbiota, and it is well recognized that immune system activity is strongly influenced by commensal bacteria (Brown and Clarke, 2016; Levy et al., 2016; Thaiss et al., 2016). While it is intuitive that bacteria in the gut would impact mucosal immune responses, the ability of gut microbes to impact immune activity in different sites, such as the bone marrow, spleen, or lungs, is less intuitive. However, an increasing number of studies demonstrate the far reaching impact of commensal microbes on immune system activity throughout the body (Brown and Clarke, 2016; Levy et al., 2016). Thus, it was determined whether stressor-induced changes in the composition of the gut microbiota were also associated with stressor-induced immunomodulation.

Stressor exposure is also well known to impact immune system activity, and the mechanisms by which this occurs are well-defined (e.g., stressor-induced glucocorticoids that can suppress key transcription factors) (Padgett and Glaser, 2003). Adrenergic receptor activation by norepinephrine or epinephrine (products of sympathetic nervous system activity) can either enhance or suppress immune system activity (Scanzano and Cosentino, 2015). However, not all of the stressor-induced enhancements of immune system functioning can be explained by adrenergic activation, and the range of mechanisms by which stressor exposure can enhance immune system activity are not well understood. We, as well as others, have hypothesized that the commensal microbiota are involved in stressor-induced immunopotentiation (Bailey et al., 2011; Maslanik, 2012). Stressor-enhanced immune

system activity has been studied in laboratory rats exposed to repeated tail shock as well as in mice exposed to the SDR stressor. Exposure to the SDR stressor significantly increases inflammatory cytokines (such as IL-1 and IL-6) in circulation and in organs such as the spleen and liver (Engler et al., 2008; Stark et al., 2002). Thus, we assessed whether stressor-induced changes in cytokine levels were associated with stressor-induced changes in the microbiota. Interestingly, it was found that the levels of IL-6 were significantly correlated with levels of *Coprococcus, Pseudobutyrovibrio*, and *Dorea* spp. Perhaps more importantly, treating mice with antibiotics to affect the microbiota attenuated the stressor-induced increase in IL-6 levels (Bailey et al., 2011). Fleshner's group observed a similar phenomenon in rats exposed to repeated tail shock; treatment with antibiotics to affect microbial community composition significantly reduced the effects of the stressor on cytokine levels in circulation. Interestingly, these effects were most evident for cytokines whose production is dependent upon activation of the inflammasome (Maslanik, 2012). Because inflammasome activation can be triggered by bacterial products, it has been suggested that commensal microbes contribute to stressor-induced immunopotentiation by activating the inflammasome (Maslanik, 2012, 2013).

Beneficial Effects of the Microbiota on Host Immunity During Stressful Stimuli

Stressor-induced increases in circulating cytokines have important implications for both behavior and immunity. For example, IL-6 in the brain has been shown to increase ambulatory exploration, digging, rearing, and locomotion. This type of behavioral profile would be protective during natural stressors in wild animals, such as predator-prey stressors (Zalcman et al., 1998). In addition to the behavioral effects of IL-6, many of the host responses to the social disruption stressor are dependent upon signaling through the IL-1 receptor. For example, we have found that anxiety-like behavior does not develop in IL-1R1$^{-/-}$ mice (Wohleb et al., 2011; Wohleb et al., 2014). In addition, SDR-induced increases in macrophage microbicidal activity fail to occur in IL-1R1$^{-/-}$ mice (Allen et al., 2012b). Of importance, social disruption-induced increases in macrophage microbicidal activity also fail to occur in germfree mice and in mice treated with antibiotics to impact gut microbiota community composition (Allen et al., 2012a). This supports the notion that gut bacteria are associated with stressor-induced increases in innate immunity and has led to studies to determine how the microbiota contribute to interactions between stressor-induced increases in macrophage activity and circulating cytokines.

Homeostatic interactions between the host and its microbiota are maintained by spacial separation between microbes (as well as microbial products) and host tissue. In the gut, this spacial separation is maintained by a thick mucous layer that contains antimicrobial compounds (including defensins, cathelicidins, and C-type lectins). Beneath the mucous layer lies the epithelial barrier comprised of a single layer of epithelial cells connected by well-defined tight junctions. The tight junctions are necessary to regulate the absorption of nutrients from the lumen of the intestines to the interior of the body, but they also form a formidable barrier to microbes. These defenses are significantly altered during a physiological stress response. For example, following repeated exposure to a water

avoidance stressor, there was a thinning of the mucous layer and a reduction of mucous-producing goblet cells which led to an increase in bacterial-epithelial interactions in the rat colon (Soderholm et al., 2002). Similar observations occurred in an acute restraint stress model in rats in which there was an elevation in mucin release and goblet cell depletion following 30 minutes of restraint (Castagliuolo et al., 1998). Each of these studies concluded enhanced mast cell activity and corticotropin-releasing hormone were the cause of the stressor-induced changed in mucous. In addition to its effects on mucous, the restraint stressor significantly reduced sIgA under either prolonged (Jarillo-Luna et al., 2007; Jarillo-Luna et al., 2008; Zoppi et al., 2012) or acute (Ponferrada et al., 2007) conditions. Restraint also had profound effects on members of the AMP family. Both CRAMP, a member of the cathelicidin family, and β-defensin 3 were significantly reduced in the epidermis following stressor exposure which led to an enhanced susceptibility to cutaneous infections (Aberg et al., 2007). These effects could be mimicked with topical corticosterone treatment. Colonic β-defensin 3 was also significantly reduced by prolonged restraint stressor exposure during *Citrobacter rodentium* challenge (Mackos et al., 2013).

In addition, it is well recognized that epithelial permeability is increased during a physiological stress response. These stressor-induced reductions in epithelial barrier integrity are associated with an ability of commensal microbes to translocate from the lumen of the intestines to the interior of the body. For example, exposure to the social disruption stressor, or to prolonged restraint stressor, significantly increases the likelihood that intestinal (and cutaneous) bacteria will translocate to secondary lymphoid organs (Bailey et al., 2006). We have recently confirmed and extended these results by demonstrating that commensal bacteria migrate from the gut to the spleen where they are found within splenic myeloid cells (namely monocytes/macrophages and neutrophils). Of importance, splenic monocytes that contain detectable, but low, levels of bacteria RNA also produce significantly higher levels of IL-1β (Lafuse et al., Under Review). This suggests that very low levels of bacterial translocation from the gut to the spleen is one mechanism by which stressor exposure leads to heightened cytokines in the blood and organs outside of the gut.

The physiological stress response serves to maximize the likelihood that an organism can adapt to and survive threatening stimuli. During aggressive confrontations, such as during the SDR stressor, the stress response increases survival by enhancing the ability of an organism to fight with or flee from an aggressive conspecific or predator. Likewise, enhanced immunity, particularly innate immunity, would increase survival if wounding or pathogen exposure occurred during the stressor. Thus, stressor-induced immunopotentiation has been suggested to be an adaptive component of the physiological stress response (Fleshner, 2013). Stressor-induced behavioral alterations are also adaptive in response to threatening stimuli. In particular, anxiety-like behavior, which is largely conceptualized as an increased state of arousal, results in hypervigilance and fearful responses (Bailey and Crawley, 2009). Under natural conditions, this would increase the likelihood of avoiding confrontations with aggressive conspecifics or with predators. The commensal microbiota plays a key role in these adaptive responses to stressor exposure through mechanisms that may be dependent upon bacterial translocation. In response to stressor exposure, the microbiota migrates from the lumen of the intestines to the interior of the body (Bailey et al., 2006). The spleen plays a role in filtering microbes from the blood, and splenic monocytes

from stressor-exposed mice contain higher levels of microbial nucleic acids than do monocytes from non-stressed control mice. These cells then produce high levels of inflammatory cytokines (such as IL-1β and IL-23) (Lafuse et al., Under Review), which can further enhance the innate immune response and also contribute to the development of anxietylike behavior, thus demonstrating the adaptive potential of stressor-induced alterations in the commensal microbiota.

Detrimental Effects of the Microbiota on Host Immunity During Stressful Stimuli

There are now several studies providing compelling evidence that microbes are involved in stressor-induced immune potentiation in the blood and spleen. However, the microbiota plays a more direct role in affecting mucosal immune responses. And in fact, diseases associated with excessive inflammatory responses in the intestines, such as the inflammatory bowel diseases, involve disrupted homeostatic interactions between the host and its gut microbiota as the result of multiple genetic and environmental factors. Studies have consistently shown that stool from patients with IBD contain different microbial community profiles than stool from non-disease controls. IBD patients have an increase in bacteria in the phylum Proteobacteria and a decrease in bacteria in the Firmicutes phylum (Hansen et al., 2010). It is not yet clear whether the differences in the gut microbiota cause, or are the result of, chronic intestinal inflammation, but animal studies clearly demonstrate that intestinal microbes can initiate and exacerbate severe colonic inflammation in susceptible individuals. Interestingly, many inflammatory diseases, including the inflammatory bowel diseases, are known to be worsened during stressful situations. For example, children with inflammatory bowel disease are more likely to have depression, anxiety, and difficulties socially, in part because they are faced with psychosocial burdens that affect their quality of life (Greenley et al., 2010; Mackner and Crandall, 2006, 2007; Mackner et al., 2006; Mackner et al., 2013). The severity of one's disease has been linked to major life stress and exposure to a perceived stressor has been shown to trigger a flare of one's disease (Bernstein et al., 2010; Mardini et al., 2004; Traue and Kosarz, 1999). These studies suggest that exposure to a stressor could exacerbate one's disease, but the exact mechanism of how this occurs in IBD is not known.

We, and others, have been testing the hypothesis that changes in the composition of the gut microbiota may contribute to intestinal inflammation. Rats exposed to chronic stress, such as chronic subordinate colony housing, often develop colonic inflammation through an adrenal-dependent mechanism (Reber et al., 2007). In our studies involving stress in mice, stressor exposure alone is often insufficient to induce pronounced colonic inflammation. However, like studies in rats (Reber et al., 2006; Reber et al., 2008; Veenema et al., 2008), exposure to stress enhances the response to inflammatory challenge in the colon. For example, we have shown that exposing mice to either the prolonged restraint stressor or to the SDR stressor significantly increases the inflammatory response to the colonic pathogen *Citrobacter rodentium* (Mackos et al., 2013; Mackos et al., 2016). This excessive inflammatory response is associated with immunopathology in the colon, and is consistent with clinical studies indicating that the symptoms of colonic diseases, such as the inflammatory bowel diseases, tend to be more severe during stressful periods. Because the prolonged restraint stressor and

the SDR stressor reduce lactobacilli levels in the colon (Galley et al., 2014a; Galley et al., 2014b), and because lactobacilli are well-known to be immunomodulatory (Hemarajata et al., 2013; Jones and Versalovic, 2009; Lin et al., 2008; Pena et al., 2005; Thomas et al., 2012), we hypothesized that stressor-induced reductions in lactobacilli were contributing to changes in the mucosal immune response. This led to a series of experiments in which mice were given probiotic lactobacilli to determine whether increasing *Lactobacillus* levels in the intestines would prevent the deleterious effects of the stressor on colonic inflammation. Administering probiotic *Lactobacillus reuteri* (ATCC23272) was shown to restore barrier function in outbred CD-1 mice exposed to the restraint stressor during *C. rodentium* challenge (Mackos et al., 2013), and prevented the exacerbating effects of the SDR stressor on colonic immunopathology in inbred C57BL/6 mice challenged with *C. rodentium* (Mackos et al., 2016). When considered together, these studies demonstrate that stressor exposure changes the composition of the microbiota and that microbes are involved with stressor-induced changes in mucosal and systemic immune responses. However, these studies were not designed to address whether stressor-induced changes in the composition of the microbiota directly lead to differences in host immunity. Because stressor exposure impacts the host at the same time it leads to changes in the composition of the gut microbiota, it is not clear from these studies whether changes in the composition of the gut microbiota alone are sufficient to significantly impact immune responses.

To address this question, we took advantage of germ-free (GF) mice, which are mice that are born and raised in sterile conditions, and thus are not colonized by any microbes. The GF mice can also be colonized by defined consortia of microbes to study the impact these microbes have on host biology. This strategy has been used to demonstrate the importance of intestinal microbes for obesity and intestinal inflammatory responses. However, to date, nobody has assessed whether transplanting the microbiota from stressor exposed animals into GF mice will have a different effect on the GF mice than transplanting microbiota from non-stressed control mice. We used microbiota transplant into GF mice to test the hypothesis that stressor-induced alterations to the microbiota were directly associated with a heightened inflammatory response to *C. rodentium* challenge.

Conventional, outbred CD-1 male mice were exposed to the prolonged restraint stressor for 6 consecutive days. At the end of stressor exposure, the contents of both the small and large intestines were aseptically removed and placed in reduced PBS under anaerobic conditions. In addition, mucosal scrapings were collected from the small and large intestines and pooled with the luminal contents. This pool, which contained both luminal as well as mucosa associated bacterial populations, contained different microbial consortia from stressor-exposed mice compared to non-stressed control mice based on results with 16s rRNA gene sequencing (Galley et al., Under Review). Thus, the intestinal sample pools were diluted 1:10,000 under anaerobic conditions and administered to germfree recipient CD-1 mice via oral gavage to colonize the intestines. Thus, germfree recipient mice were colonized with microbiota from non-stressed control or from stressor-exposed conventional mice. On the day following colonization, the mice were challenged with the colonic pathogen, *C. rodentium*. Interestingly, *C. rodentium* challenge resulted in greater colonic inflammation and greater colon mass in mice that received microbiota from stressor-exposed donors compared to mice that received microbiota from non-stressed controls. These data indicate

that stressor-induced changes in the composition of the gut microbiota play a role in the stressor-induced exacerbation of *C. rodentium*-induced colonic inflammation. These findings are consistent with the hypothesis that intestinal diseases, such as irritable bowel syndrome and the inflammatory bowel diseases, are exacerbated during stressful periods in part because of stressor-induced alterations in the composition of the gut microbiota.

Excessive inflammatory responses in the intestines lead to immunopathology. Thus, the propensity of the stress response (and changes in the microbiota) to lead to excessive inflammatory responses in the intestines would not be considered an adaptive component of the stress response. However, it should be noted that in many, but not all, paradigms the excessive inflammatory state needs to be triggered, such as through the induction of a pathogen (like *C. rodentium* or enteroinvasive *E. coli*) or chemical (such as DSS). In the absence of such stimuli, the prolonged restraint stressor or social disruption stressor does not lead to an excessive inflammatory response in the intestines of mice (Bailey, unpublished observations). Instead, the intestines are thought to be in a state of “physiological inflammation”, which is characterized as a low grade, and presumably more well controlled, inflammatory state that actually helps to maintain homeostasis between the host and its microbiota (Collins, 2001). Excessive, tissue-damaging inflammatory responses are thought to occur when this homeostasis is disrupted.

Conclusions

Since the emergence of the field of PsychoNeuroImmunology, there has been an intense interest in understanding the ways in which the immune system interacts with the nervous system. While there has been a recent surge in studies focused on determining the impact that the immune system can have on the brain and on behavior, some of the original studies in the field focused on determining the impact that the brain and behavior can have on immune system functioning. These studies were triggered by findings that immune system activation could be conditioned through classical conditioning paradigms, and also by studies showing that body’s physiological response to psychosocial stressors was associated with various diseases and conditions. As more mechanistic studies were developed, it became realized that neuroendocrine hormones could impact the functioning of the immune system. The effects of the catecholamines (primarily norepinephrine and epinephrine) and glucocorticoid hormones (primarily cortisol and corticosterone) on immune system function were heavily studied, and many of the effects that they can have on immunity are now well described. In large part, these neurotransmitters and hormones suppress the functioning of the immune system, and many of the mechanisms by which this occurs, such as through the suppression of key transcription factors (like NF-κB). However, mechanisms by which the immune system can be enhanced during stressful periods are not as well understood. We, and others, hypothesize that this is because stressor-induced immunopotentiation not only involves potential immune-enhancive effects of hormones and neurotransmitters, but also involves the commensal microbiota.

The commensal microbiota are well known to affect both mucosal immune responses, and immune responses in distant compartments, such as the spleen. Multiple studies now indicate that the commensal microbiota are involved in stressor-induced immunopotentiation

in both of these sites. Throughout evolution, these effects would have been adaptive. For example, in a predator/prey situation where physical confrontations are possible, increased innate immunity and increased fearfulness of the situation would help to recover from and avoid similar situations. However, if left uncontrolled, the stressor-induced increases in immune system activity can be maladaptive, and can lead to tissue-damaging immune responses. It is of interest that commensal microbes are key players in both of the outcomes.

The mechanisms by which the microbiota contribute to stressor-induced immunopotentiation are not completely understood. It is possible that direct contact between the microbiota and the host are responsible for the increased immune activity. In the gut, the commensal gut microbiota are separated from the host epithelium by a thick layer of mucous and the secretion of antimicrobial peptides. Because stressor exposure can affect both mucous and antimicrobial peptide production, it is possible that this allows commensal microbes to come into contact with the gut epithelium to elicit an immune response. Bacterial contact with the gut epithelium is also a necessary step in the ability of the microbes to translocate from the intestines to the interior of the body, where they are filtered from circulation from organs such as the spleen. The translocation of microbes, and/or their products, can prime the innate immune system for enhanced reactivity. It is likely that neuroendocrine hormones and commensal microbes have synergistic, and/or counter-regulatory effects on immune cell functioning. However, it is not yet known how the microbiota influence neuroendocrine effects on immune cell activity. This topic warrants further investigation in future studies.

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References

Aberg KM, Radek KA, Choi EH, Kim DK, Demerjian M, Hupe M, Kerbleski J, Gallo RL, Ganz T, Mauro T, Feingold KR, Elias PM. Psychological stress downregulates epidermal antimicrobial peptide expression and increases severity of cutaneous infections in mice. The Journal of clinical investigation. 2007; 117:3339–3349. [PubMed: 17975669]

Allen RG, Lafuse WP, Galley JD, Ali MM, Ahmer BM, Bailey MT. The intestinal microbiota are necessary for stressor-induced enhancement of splenic macrophage microbicidal activity. Brain, behavior, and immunity. 2012a; 26:371–382.

Allen RG, Lafuse WP, Powell ND, Webster Marketon JI, Stiner-Jones LM, Sheridan JF, Bailey MT. Stressor-induced increase in microbicidal activity of splenic macrophages is dependent upon peroxynitrite production. Infection and immunity. 2012b; 80:3429–3437. [PubMed: 22825446]

Antonopoulos DA, Huse SM, Morrison HG, Schmmidt TM, Sogin ML, Young VB. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infection and immunity. 2009; 77:2367–2375. [PubMed: 19307217]

Aoki-Yoshida A, Aoki R, Moriya N, Goto T, Kubota Y, Toyoda A, Takayama Y, Suzuki C. Omics Studies of the Murine Intestinal Ecosystem Exposed to Subchronic and Mild Social Defeat Stress. Journal of proteome research. 2016

Bailey, KR., Crawley, JN. Anxiety-Related Behaviors in Mice. In: Buccafusco, JJ., editor. Methods of Behavior Analysis in Neuroscience. 2nd. Boca Raton (FL): 2009.

Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Developmental psychobiology. 1999; 35:146–155. [PubMed: 10461128]

Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain, behavior, and immunity. 2011; 25:397–407.

Bailey MT, Dowd SE, Parry NM, Galley JD, Schauer DB, Lyte M. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. Infection and immunity. 2010; 78:1509–1519. [PubMed: 20145094]

Bailey MT, Engler H, Sheridan JF. Stress induces the translocation of cutaneous and gastrointestinal microflora to secondary lymphoid organs of C57BL/6 mice. Journal of neuroimmunology. 2006; 171:29–37. [PubMed: 16253348]

Bailey MT, Lubach GR, Coe CL. Prenatal stress alters bacterial colonization of the gut in infant monkeys. Journal of pediatric gastroenterology and nutrition. 2004; 38:414–421. [PubMed: 15085020]

Bangsgaard Bendtsen KM, Krych L, Sorensen DB, Pang W, Nielsen DS, Josefsen K, Hansen LH, Sorensen SJ, Hansen AK. Gut microbiota composition is correlated to grid floor induced stress and behavior in the BALB/c mouse. PloS one. 2012; 7:e46231. [PubMed: 23056268]

Bansal T, Englert D, Lee J, Hegde M, Wood TK, Jayaraman A. Differential effects of epinephrine, norepinephrine, and indole on Escherichia coli O157:H7 chemotaxis, colonization, and gene expression. Infection and immunity. 2007; 75:4597–4607. [PubMed: 17591798]

Berg RD. The indeginous gastrointestinal microflora. Trends Microbiol. 1996; 4:430–435. [PubMed: 8950812]

Bernstein CN, Singh S, Graff LA, Walker JR, Miller N, Cheang M. A prospective population-based study of triggers of symptomatic flares in IBD. The American journal of gastroenterology. 2010; 105:1994–2002. [PubMed: 20372115]

Bharwani A, Mian MF, Foster JA, Surette MG, Bienenstock J, Forsythe P. Structural & functional consequences of chronic psychosocial stress on the microbiome & host. Psychoneuroendocrinology. 2016; 63:217–227. [PubMed: 26479188]

Brown RL, Clarke TB. The regulation of host defences to infection by the microbiota. Immunology. 2016

Campos-Rodriguez R, Godinez-Victoria M, Abarca-Rojano E, Pacheco-Yepez J, Reyna-Garfias H, Barbosa-Cabrera RE, Drago-Serrano ME. Stress modulates intestinal secretory immunoglobulin A. Frontiers in integrative neuroscience. 2013; 7:86. [PubMed: 24348350]

Castagliuolo I, Wershil BK, Karalis K, Pasha A, Nikulasson ST, Pothoulakis C. Colonic mucin release in response to immobilization stress is mast cell dependent. The American journal of physiology. 1998; 274:G1094–G1100. [PubMed: 9696710]

Clements WD, Parks R, Erwin P, Halliday MI, Barr J, Rowlands BJ. Role of the gut in the pathophysiology of extrahepatic biliary obstruction. Gut. 1996; 39:587–593. [PubMed: 8944570]

Collins SM. Stress and the Gastrointestinal Tract IV. Modulation of intestinal inflammation by stress: basic mechanisms and clinical relevance. American journal of physiology. Gastrointestinal and liver physiology. 2001; 280:G315–G318. [PubMed: 11171612]

Da Silva S, Robbe-Masselot C, Ait-Belgnaoui A, Mancuso A, Mercade-Loubiere M, Salvador-Cartier C, Gillet M, Ferrier L, Loubiere P, Dague E, Theodorou V, Mercier-Bonin M. Stress disrupts intestinal mucus barrier in rats via mucin O-glycosylation shift: prevention by a probiotic treatment. American journal of physiology. Gastrointestinal and liver physiology. 2014; 307:G420–G429. [PubMed: 24970779]

De Palma G, Blennerhassett P, Lu J, Deng Y, Park AJ, Green W, Denou E, Silva MA, Santacruz A, Sanz Y, Surette MG, Verdu EF, Collins SM, Bercik P. Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nature communications. 2015; 6:7735.

Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008:6.

Dinan TG, Cryan JF. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology. 2012; 37:1369–1378. [PubMed: 22483040]

Drasar BS, Shiner M, McLeod GM. Studies on the intestinal flora. I. The baterial flora of the gastrointestinal tract in healthy and achlorydic persons. Gastroenterology. 1969; 56:71–79. [PubMed: 4885396]

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science. 2005; 308:1635–1638. [PubMed: 15831718]

Engler H, Bailey MT, Engler A, Stiner-Jones LM, Quan N, Sheridan JF. Interleukin-1 receptor type 1-deficient mice fail to develop social stress-associated glucocorticoid resistance in the spleen. Psychoneuroendocrinology. 2008; 33:108–117. [PubMed: 18037570]

Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. Science. 2002; 298:1424–1427. [PubMed: 12434060]

Fleshner M. Stress-evoked sterile inflammation, danger associated molecular patterns (DAMPs), microbial associated molecular patterns (MAMPs) and the inflammasome. Brain, behavior, and immunity. 2013; 27:1–7.

Freestone P, Lyte M. Stress and microbial endocrinology: prospects for ruminant nutrition. Animal : an international journal of animal bioscience. 2010; 4:1248–1257. [PubMed: 22444620]

Freestone PP, Williams PH, Haigh RD, Maggs AF, Neal CP, Lyte M. Growth stimulation of intestinal commensal Escherichia coli by catecholamines: a possible contributory factor in trauma-induced sepsis. Shock. 2002; 18:465–470. [PubMed: 12412628]

Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J, Kumar PS, Lyte M, Bailey MT. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. BMC microbiology. 2014a; 14:189. [PubMed: 25028050]

Galley JD, Yu Z, Kumar P, Dowd SE, Lyte M, Bailey MT. The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. Gut microbes. 2014b; 5:748–760. [PubMed: 25536463]

Golubeva AV, Crampton S, Desbonnet L, Edge D, O'Sullivan O, Lomasney KW, Zhdanov AV, Crispie F, Moloney RD, Borre YE, Cotter PD, Hyland NP, O'Halloran KD, Dinan TG, O'Keeffe GW, Cryan JF. Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. Psychoneuroendocrinology. 2015; 60:58–74. [PubMed: 26135201]

Greenley RN, Hommel KA, Nebel J, Raboin T, Li SH, Simpson P, Mackner L. A meta-analytic review of the psychosocial adjustment of youth with inflammatory bowel disease. Journal of pediatric psychology. 2010; 35:857–869. [PubMed: 20123705]

Hansen J, Gulati A, Sartor RB. The role of mucosal immunity and host genetics in defining intestinal commensal bacteria. Current opinion in gastroenterology. 2010; 26:564–571. [PubMed: 20871399]

Hemarajata P, Gao C, Pflughoeft KJ, Thomas CM, Saulnier DM, Spinler JK, Versalovic J. Lactobacillus reuteri-specific immunoregulatory gene rsiR modulates histamine production and immunomodulation by Lactobacillus reuteri. Journal of bacteriology. 2013; 195:5567–5576. [PubMed: 24123819]

Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012; 336:1268–1273. [PubMed: 22674334]

Hughes DT, Clarke MB, Yamamoto K, Rasko DA, Sperandio V. The QseC adrenergic signaling cascade in Enterohemorrhagic E. coli (EHEC). PLoS pathogens. 2009; 5:e1000553. [PubMed: 19696934]

Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, Yu RT, Shelton JM, Richardson JA, Repa JJ, Mangelsdorf DJ, Kliewer SA. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:3920–3925. [PubMed: 16473946]

Jarillo-Luna A, Rivera-Aguilar V, Garfias HR, Lara-Padilla E, Kormanovsky A, Campos-Rodriguez R. Effect of repeated restraint stress on the levels of intestinal IgA in mice. Psychoneuroendocrinology. 2007; 32:681–692. [PubMed: 17590521]

Jarillo-Luna A, Rivera-Aguilar V, Martinez-Carrillo BE, Barbosa-Cabrera E, Garfias HR, Campos-Rodriguez R. Effect of restraint stress on the population of intestinal intraepithelial lymphocytes in mice. Brain, behavior, and immunity. 2008; 22:265–275.

Jasarevic E, Howerton CL, Howard CD, Bale TL. Alterations in the Vaginal Microbiome by Maternal Stress Are Associated With Metabolic Reprogramming of the Offspring Gut and Brain. Endocrinology. 2015; 156:3265–3276. [PubMed: 26079804]

Jones SE, Versalovic J. Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. BMC microbiology. 2009; 9:35. [PubMed: 19210794]

Lenz HJ. Regulation of small intestinal and pancreaticobiliary functions by CRF. Annals of the New York Academy of Sciences. 1993; 697:254–259. [PubMed: 8257015]

Lenz HJ, Silverman TA, Messmer AG, Zimmerman FG. Increased sympathetic outflow to the gut by cerebral CGRP inhibits duodenal, pancreatic, small intestinal, and biliary functions. Annals of the New York Academy of Sciences. 1992; 657:522–524. [PubMed: 1637112]

Lenz HJ, Zimmerman FG, Messmer B. Regulation of canine gallbladder motility by brain peptides. Gastroenterology. 1993; 104:1678–1685. [PubMed: 8500726]

Levy M, Thaiss CA, Elinav E. Metabolites: messengers between the microbiota and the immune system. Genes & development. 2016; 30:1589–1597. [PubMed: 27474437]

Lin YP, Thibodeaux CH, Pena JA, Ferry GD, Versalovic J. Probiotic Lactobacillus reuteri suppress proinflammatory cytokines via c-Jun. Inflammatory bowel diseases. 2008; 14:1068–1083. [PubMed: 18425802]

Lorenzo-Zuniga V, Bartoli R, Planas R, Hofmann AF, Vinado B, Hagey LR, Hernandez JM, Mane J, Alvarez MA, Ausina V, Gassull MA. Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. Hepatology. 2003; 37:551–557. [PubMed: 12601352]

Lyte M. The role of microbial endocrinology in infectious disease. The Journal of endocrinology. 1993; 137:343–345. [PubMed: 8371072]

Lyte M. Microbial endocrinology and infectious disease in the 21st centurt. Trends Microbiol. 2004; 12:14–20. [PubMed: 14700547]

Lyte M. The microbial organ in the gut as a driver of homeostasis and disease. Medical hypotheses. 2010; 74:634–638. [PubMed: 19900764]

Lyte M. The effect of stress on microbial growth. Animal health research reviews / Conference of Research Workers in Animal Diseases. 2014; 15:172–174.

Lyte M. Microbial Endocrinology in the Pathogenesis of Infectious Disease. Microbiology spectrum. 2016a:4.

Lyte M. Microbial Endocrinology: An Ongoing Personal Journey. Advances in experimental medicine and biology. 2016b; 874:1–24. [PubMed: 26589212]

Lyte M, Arulanandam B, Nguyen K, Frank C, Erickson A, Francis D. Norepinephrine induced growth and expression of virulence associated factors in enterotoxigenic and enterohemorrhagic strains of Escherichia coli. Advances in experimental medicine and biology. 1997; 412:331–339. [PubMed: 9192038]

Lyte M, Bailey MT. Neuroendocrine-bacterial interactions in a neurotoxin-induced model of trauma. The Journal of surgical research. 1997; 70:195–201. [PubMed: 9245571]

Lyte M, Ernst S. Catecholamine induced growth of gram negative bacteria. Life Sci. 1992; 50:203–212. [PubMed: 1731173]

Lyte M, Vulchanova L, Brown DR. Stress at the intestinal surface: catecholamines and mucosa-bacteria interactions. Cell and tissue research. 2011; 343:23–32. [PubMed: 20941511]

Mackner LM, Crandall WV. Brief report: psychosocial adjustment in adolescents with inflammatory bowel disease. Journal of pediatric psychology. 2006; 31:281–285. [PubMed: 15802606]

Mackner LM, Crandall WV. Psychological factors affecting pediatric inflammatory bowel disease. Current opinion in pediatrics. 2007; 19:548–552. [PubMed: 17885473]

Mackner LM, Crandall WV, Szigethy EM. Psychosocial functioning in pediatric inflammatory bowel disease. Inflammatory bowel diseases. 2006; 12:239–244. [PubMed: 16534426]

Mackner LM, Greenley RN, Szigethy E, Herzer M, Deer K, Hommel KA. Psychosocial issues in pediatric inflammatory bowel disease: report of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition. Journal of pediatric gastroenterology and nutrition. 2013; 56:449–458. [PubMed: 23287808]

Mackos AR, Eubank TD, Parry NM, Bailey MT. Probiotic Lactobacillus reuteri attenuates the stressor-enhanced severity of Citrobacter rodentium infection. Infection and immunity. 2013; 81:3253–3263. [PubMed: 23798531]

Mackos AR, Galley JD, Eubank TD, Easterling RS, Parry NM, Fox JG, Lyte M, Bailey MT. Social stress-enhanced severity of Citrobacter rodentium-induced colitis is CCL2-dependent and attenuated by probiotic Lactobacillus reuteri. Mucosal immunology. 2016; 9:515–526. [PubMed: 26422754]

Mantis NJ. Rediscovering IgA. Mucosal immunology. 2011; 4:588–589. [PubMed: 22005880]

Mantis NJ, Rol N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal immunology. 2011; 4:603–611. [PubMed: 21975936]

Mardini HE, Kip KE, Wilson JW. Crohn's disease: a two-year prospective study of the association between psychological distress and disease activity. Digestive diseases and sciences. 2004; 49:492–497. [PubMed: 15139504]

Maslanik T, Mahaffey L, Tannura K, Beninson L, Greenwood BN, Fleshner M. The inflammasome and danger associated molecular patterns (DAMPs) are implicated in cytokine and chemokine responses following stressor exposure. Brain, behavior, and immunity. 2013; 28:54–62.

Maslanik T, Tannura K, Mahaffey L, et al. Commensal bacteria and MAMPs are necessary for stress-induced increases in IL-1β and IL-18 but not IL-6, IL-10 or MCP-1. PloS one. 2012; 7:e50636. [PubMed: 23236381]

McRae S, Younger K, Thompson DG, Wingate DL. Sustained mental stress alters human jejunal motor activity. Gut. 1982; 23:404–409. [PubMed: 7076017]

Messmer B, Zimmerman FG, Lenz HJ. Regulation of exocrine pancreatic secretion by cerebral TRH and CGRP: role of VIP, muscarinic, and adrenergic pathways. The American journal of physiology. 1993; 264:G237–G242. [PubMed: 8447406]

Moloney RD, Johnson AC, O'Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. CNS neuroscience & therapeutics. 2016; 22:102–117. [PubMed: 26662472]

Moreira CG, Russell R, Mishra AA, Narayanan S, Ritchie JM, Waldor MK, Curtis MM, Winter SE, Weinshenker D, Sperandio V. Bacterial Adrenergic Sensors Regulate Virulence of Enteric Pathogens in the Gut. mBio. 2016:7.

Moreira CG, Sperandio V. Interplay between the QseC and QseE bacterial adrenergic sensor kinases in Salmonella enterica serovar Typhimurium pathogenesis. Infection and immunity. 2012; 80:4344–4353. [PubMed: 23027532]

Nyuyki KD, Beiderbeck DI, Lukas M, Neumann ID, Reber SO. Chronic subordinate colony housing (CSC) as a model of chronic psychosocial stress in male rats. PloS one. 2012; 7:e52371. [PubMed: 23300653]

O'Mahoney SM, Marchesi JR, Scully P, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biological psychiatry. 2009; 65:263–267.

Ostaff MJ, Stange EF, Wehkamp J. Antimicrobial peptides and gut microbiota in homeostasis and pathology. EMBO molecular medicine. 2013; 5:1465–1483. [PubMed: 24039130]

Padgett DA, Glaser R. How stress influences the immune response. Trends in immunology. 2003; 24:444–448. [PubMed: 12909458]

Pena JA, Rogers AB, Ge Z, Ng V, Li SY, Fox JG, Versalovic J. Probiotic Lactobacillus spp. diminish Helicobacter hepaticus-induced inflammatory bowel disease in interleukin-10-deficient mice. Infection and immunity. 2005; 73:912–920. [PubMed: 15664933]

Ponferrada A, Caso JR, Alou L, Colon A, Sevillano D, Moro MA, Lizasoain I, Menchen P, Gomez-Lus ML, Lorenzo P, Cos E, Leza JC, Menchen L. The role of PPARgamma on restoration of colonic homeostasis after experimental stress-induced inflammation and dysfunction. Gastroenterology. 2007; 132:1791–1803. [PubMed: 17484875]

Pullinger GD, Carnell SC, Sharaff FF, et al. Norepinephrine augments *Salmonella enterica*-induced enteritis in a manner associated with increased net replication but independent of the putative adrenergic sensor kinasa QseC and QseE. Infection and immunity. 2010; 78:372–380. [PubMed: 19884332]

Pusceddu MM, El Aidy S, Crispie F, O'Sullivan O, Cotter P, Stanton C, Kelly P, Cryan JF, Dinan TG. N-3 Polyunsaturated Fatty Acids (PUFAs) Reverse the Impact of Early-Life Stress on the Gut Microbiota. PloS one. 2015; 10:e0139721. [PubMed: 26426902]

Reber SO, Birkeneder L, Veenema AH, Obermeier F, Falk W, Straub RH, Neumann ID. Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. Endocrinology. 2007; 148:670–682. [PubMed: 17110427]

Reber SO, Obermeier F, Straub RH, Falk W, Neumann ID. Chronic intermittent psychosocial stress (social defeat/overcrowding) in mice increases the severity of an acute DSS-induced colitis and impairs regeneration. Endocrinology. 2006; 147:4968–4976. [PubMed: 16794011]

Reber SO, Obermeier F, Straub RH, Veenema AH, Neumann ID. Aggravation of DSS-induced colitis after chronic subordinate colony (CSC) housing is partially mediated by adrenal mechanisms. Stress. 2008; 11:225–234. [PubMed: 18465469]

Reyna-Garfias H, Miliar A, Jarillo-Luna A, Rivera-Aguilar V, Pacheco-Yepez J, Baeza I, Campos-Rodriguez R. Repeated restraint stress increases IgA concentration in rat small intestine. Brain, behavior, and immunity. 2010; 24:110–118.

Sakuma K, Funabashi H, Matsuoka H, Saito M. Potential use ot *Lactobacillus* cell density in feces as a non-invasive bio-indicator for evaluating environmental stress during mouse breeding. Biocontrol Sci. 2013; 18:101–104. [PubMed: 23796642]

Salzman NH. Paneth cell defensins and the regulation of the microbiome: detente at mucosal surfaces. Gut microbes. 2010; 1:401–406. [PubMed: 21468224]

Sandrini SM, Shergill R, Woodward J, Muralikuttan R, Haigh RD, Lyte M, Freestone PP. Elucidation of the mechanism by which catecholamine stress hormones liberate iron from the innate immune defense proteins transferrin and lactoferrin. Journal of bacteriology. 2010; 192:587–594. [PubMed: 19820086]

Scanzano A, Cosentino M. Adrenergic regulation of innate immunity: a review. Frontiers in pharmacology. 2015; 6:171. [PubMed: 26321956]

Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. Physiological reviews. 2010; 90:859–904. [PubMed: 20664075]

Shigeshiro M, Tanabe S, Suzuki T. Repeated exposure to water immersion stress reduced the Muc2 gene level in the rat colon via two distinct mechanisms. Brain, behavior, and immunity. 2012; 26:1061–1065.

Soderholm JD, Yang PC, Ceponis P, Vohra A, Riddell R, Sherman PM, Perdue MH. Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine. Gastroenterology. 2002; 123:1099–1108. [PubMed: 12360472]

Stanghellini V, Malagelada JR, Zinsmeister AR, Go VL, Kao PC. Stress-induced gastroduodenal motor disturbances in humans: possible humoral mechanisms. Gastroenterology. 1983; 85:83–91. [PubMed: 6303893]

Stanghellini V, Malagelada JR, Zinsmeister AR, Go VL, Kao PC. Effect of opiate and adrenergic blockers on the gut motor response to centrally acting stimuli. Gastroenterology. 1984; 87:1104–1113. [PubMed: 6090258]

Stark JL, Avitsur R, Hunzeker J, Padgett DA, Sheridan JF. Interleukin-6 and the development of social disruption-induced glucocorticoid resistance. Journal of neuroimmunology. 2002; 124:9–15. [PubMed: 11958817]

Stearns JC, Lynch MD, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, Croitoru K, Moreno-Hagelsieb G, Neufeld JD. Bacterial biogeography of the human digestive tract. Scientific reports. 2011; 1:170. [PubMed: 22355685]

Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, Fagarasan S. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:1981–1986. [PubMed: 14766966]

Tache Y, Martinez V, Million M, Wang L. Stress and the gastrointestinal tract III. Stress-related alterations of gut motor function: role of brain corticotropin-releasing factor receptors. American journal of physiology. Gastrointestinal and liver physiology. 2001; 280:G173–G177. [PubMed: 11208537]

Tache Y, Perdue MH. Role of peripheral CRF signalling pathways in stress-related alterations of gut motibility and mucosal function. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2004; 16:137–142. [PubMed: 15066020]

Tannock GW, Savage DC. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. Infection and immunity. 1974; 9:591–598. [PubMed: 4593471]

Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. Nature. 2016; 535:65–74. [PubMed: 27383981]

Thomas CM, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, Britton RA, Kalkum M, Versalovic J. Histamine derived from probiotic Lactobacillus reuteri suppresses TNF via modulation of PKA and ERK signaling. PloS one. 2012; 7:e31951. [PubMed: 22384111]

Traue HC, Kosarz P. Everyday stress and Crohn's disease activity: a time series analysis of 20 single cases. International journal of behavioral medicine. 1999; 6:101–119. [PubMed: 16250682]

Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell host & microbe. 2008; 3:213–223. [PubMed: 18407065]

Veenema AH, Reber SO, Selch S, Obermeier F, Neumann ID. Early life stress enhances the vulnerability to chronic psychosocial stress and experimental colitis in adult mice. Endocrinology. 2008; 149:2727–2736. [PubMed: 18308845]

Vlisidou I, Lyte M, van Diemen PM, Hawes P, Monaghan P, Wallis TS, Stevens MP. The neuroendocrine stress hormone norepinephrine augments Escherichia coli O157:H7-induced enteritis and adherence in a bovine ligated ileal loop model of infection. Infection and immunity. 2004; 72:5446–5451. [PubMed: 15322043]

Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF. beta-Adrenergic receptor antagonism prevents anxietylike behavior and microglial reactivity induced by repeated social defeat. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011; 31:6277–6288. [PubMed: 21525267]

Wohleb ES, Patterson JM, Sharma V, Quan N, Godbout JP, Sheridan JF. Knockdown of interleukin-1 receptor type-1 on endothelial cells attenuated stress-induced neuroinflammation and prevented anxiety-like behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014; 34:2583–2591. [PubMed: 24523548]

Yang H, Yuan PQ, Wang L, Tache Y. Activation of the parapyramidal region in the vetral medulla stimulates gastric acid secretion through vagal pathways in rats. Neuroscience. 2000; 95:773–779. [PubMed: 10670444]

Zalcman S, Murray L, Dyck DG, Greenberg AH, Nance DM. Interleukin-2 and -6 induce behavioral-activating effects in mice. Brain research. 1998; 811:111–121. [PubMed: 9804916]

Zhao X, Yin J, Wang L, Chen JD. Diffused and sustained inhibitory effects of intestinal electrical stimulation on intestinal motility mediated via sympathetic pathway. Neuromodulation : journal of the International Neuromodulation Society. 2014; 17:373–379. discussion 380. [PubMed: 23924055]

Zijlmans MA, Korpela K, Riksen-Walraven JM, de Vos WM, de Weerth C. Maternal prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology. 2015; 53:233–245. [PubMed: 25638481]

Zoppi S, Madrigal JL, Perez-Nievas BG, Marin-Jimenez I, Caso JR, Alou L, Garcia-Bueno B, Colon A, Manzanares J, Gomez-Lus ML, Menchen L, Leza JC. Endogenous cannabinoid system regulates intestinal barrier function in vivo through cannabinoid type 1 receptor activation. American journal of physiology. Gastrointestinal and liver physiology. 2012; 302:G565–G571. [PubMed: 22135307]

Highlights

- Stressor exposure can lead to community-wide changes of the commensal microbiota
- Stress-induced microbial shifts are due to hormones and antimicrobial agents
- The microbiota can have beneficial and detrimental effects on host immune function