The effects of psychological stressors on the intestinal microbiota

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The stress response affects virtually every organ in the body and constitutes a coordinated behavioral and physiological response to potentially threatening stimuli that may be physiological or psychological in nature. There is a substantial amount of research focusing on how the stress response affects health, but relatively few studies have focused on the ability of the stress response to affect indigenous populations of bacteria in the intestines, referred to as the intestinal microbiota. Research from our lab, and from others, have demonstrated that psychological stressors early in the life span significantly changes the levels of different types of microbiota that are shed from the intestines in the stool. In our studies, stress in young rhesus monkeys, and even in the prenatal period, led to a significant reduction in the levels of lactobacilli and bifidobacteria shed in the stool. In rodents, prolonged restraint stress resulted in a significant overgrowth of aerobic microbiota, particularly Gram-negative aerobes, in the intestines. Interestingly, there is increasing evidence that alterations in the microbiota are associated with a variety of diseases that are known to be exacerbated during periods of psychological stress, including irritable bowel syndrome and the inflammatory bowel diseases. Thus, our data provide a compelling rationale to test the hypothesis that stress-induced exacerbations of intestinal diseases are in part due to stress-induced alterations of the microbiota.

Key words: stress; maternal separation; prenatal stress; microbiota; Lactobacillus; Bifidobacteria; Macaca mulatta

INTRODUCTION

The human body harbors an enormous array of microbes that even in the healthy host outnumbers cells of the body by a factor of 10 (i.e., approximately 10^{14} bacterial cells to 10^{13} human cells). These bacteria colonize all external surfaces of the body, including the skin, oral and nasal cavities, upper respiratory tract, and urinary tract. The gastrointestinal tract, however, is the main reservoir of bacteria and harbors roughly 90% of these indigenous microbes, which are generally referred to as the microbiota. New molecular analyses of the intestinal microbiota have suggested that the microbiota belong to approximately 1,800 genera with up to 15,000–36,000 different individual species (24, 25). Thus, it is not surprising that the microbial genome within a human is estimated to contain more than 100 times as many genes as the human genome alone (31).

Historically, the microbiota were thought to be opportunistic colonizers with the capacity to cause disease when altered or introduced to the interior of the body. However, over time this view has changed and it is now recognized that the microbiota are true symbiotic organisms that have many beneficial effects on the host. For example, many metabolic activities in the intestines are derived from the microbiota, such as the synthesis of vitamin K and vitamin B complex and the breakdown of precarcinogens and carcinogens (65). Moreover, data now indicate that the microbiota are associated with obesity, with obese animals found to have higher levels of Firmicutes and lower levels of Bacteroidetes (45, 73). Perhaps not surprisingly, many gastrointestinal diseases, including irritable bowel syndrome (IBS) and the inflammatory bowel diseases (IBD) (55) have been linked to alterations in microbiota (58).

The intestinal microbiota are also known to play a very important role in host defense against intestinal pathogens. This is partly due to the microbiota’s ability to stimulate and enhance mucosal immunity; mice lacking an intact microbiota have several immune deficiencies, such as reduced levels of serum immunoglobulins, smaller Peyer’s patches, fewer intraepithelial lymphocytes, and a reduced capacity to produce cytokines (65). It is important to point out that introducing microbes into the germ free mice restores many of these immune deficiencies (33, 67, 74, 75). The microbiota are also able to protect against diseases.

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independently of their effects on the immune system through colonization exclusion. This exclusion refers to the ability of the normal indigenous populations of microbes to inhibit the attachment and growth of invading pathogens, as evidenced through in vivo studies and in vitro modeling. For example, members of the genera *Bifidobacterium* and *Lactobacillus* have been shown to create a physical barrier to colonization by enteropathogenic *Escherichia coli*, *Yersinia pseudotuberculosis*, and *Salmonella typhimurium* (6, 10, 11).

The idea that intestinal microbes can promote health is not new; Ely Metchnikoff hypothesized nearly 100 years ago that lactic acid bacteria (such as the lactobacilli) had beneficial effects on the host (11). As research progressed, it became evident that ingesting probiotic strains of bacteria, such as the bifidobacteria and lactobacilli, could enhance intestinal health and reduce the ability of pathogens to cause disease. And, it is now known that the maintenance of high levels of these microbes in the intestines is important for maintaining health. Despite this knowledge, relatively few studies have focused on the natural physiological mechanisms that affect the levels of these types of probiotic bacteria in the intestines. Our studies indicate that psychological stressors can influence the numbers of lactobacilli and bifidobacteria in the intestines, potentially leaving the host susceptible to enteric infections.

**PSYCHONEUROIMMUNOLOGY:**

**IMPACT OF STRESSOR EXPOSURE ON IMMUNITY**

Multidirectional communications exist between the central nervous system, endocrine system, and individual organ systems, such that the functioning of one can be detected by and influence the functioning of the others. While the importance of these types of interactions are widely acknowledged, relatively little research is focused on studying complex interactions between systems of the body. However, some multidisciplinary fields of study have begun to define how different systems of the body interact. For example, research in the field of PsychoNeuroImmunology (PNI), is focused on interactions between the nervous, endocrine, and immune systems, with one main emphasis of study focusing on how psychological stressors influence the functioning of the immune system. Psychological stress is a process in which a stimulus (i.e., a stressor) is perceived as being potentially threatening, and leads to behavioral and physiological responses (collectively called the stress response) that are meant to help the body cope and adapt to the stressor (17).

While the stress response developed to help the body cope with the demands being placed on it, the field of PNI has provided ample evidence that exposure to psychological stressors, and thus the stress response, either enhances or directly contributes to the severity of, or susceptibility to, a variety of diseases. For example, individuals reporting higher levels of stress in their daily lives are more likely to develop clinical symptoms during experimental respiratory viral infection (16). This association is thought to be due to stressor-induced immunomodulation, since stressful periods are also associated with reduced immune-responsiveness to several different types of vaccinations (32, 38, 41). Studies utilizing laboratory animals have confirmed the impact that stressors have on immune responsiveness and have identified some of the pathways through which the stress response affects the immune system. For example, stressor-induced elevations in corticosterone [one of the products of the stress-responsive hypothalamic-pituitary adrenal (HPA) axis] have been found to suppress lymphocyte trafficking and cytokine production during influenza viral infection (19, 34) as well as antigen processing and presentation by dendritic cells (68, 71, 72). Stressor-induced activation of the sympathetic nervous system (SNS) is also known to affect the immune system; adrenergic signaling is responsible for stressor-induced suppression of cytotytic CD8+ T cell responses during experimental influenza viral infection (20) and the CD4+ T cell response to experimental *Listeria monocytogenes* infection through a β1-adrenergic receptor mediated mechanism (8).

The immune system is not the only player in the infectious process that is affected during a stress response. Microbes themselves can also be affected by exposure to stress-induced hormones, such as the catecholamines. There are now several studies, and an emerging field of research coined Microbial Endocrinology, demonstrating that catecholamines can enhance the growth of many different types of microbes (26, 27, 48). For example, bacteria ranging from Gram-negative facultative anaerobes, such as *E. coli*, to Gram-positive anaerobes, like *Peptostreptococcus anaerobius* have been shown to have enhanced growth upon exposure to catecholamines (27, 28). In addition to growth, the expression of many different types of virulence factors, like pili and secretory toxins, have been shown to be enhanced in pathogens after exposure to catecholamines, thus increasing the severity of disease when catecholamine levels are high (49, 51). These data have contributed to our overlying hypothesis that exposure to psychological stressors can affect the indigenous
microbial populations in the body, with a particular focus on intestinal microbiota.

**STRESS-INDUCED ALTERATIONS IN INTESTINAL MICROBIOTA**

The intestinal microbiota form a stable ecosystem by colonizing virtually every niche in the lower intestines. And, when these populations remain stable, they have many beneficial effects on their host (54). This stability, however, has been shown to be disrupted by environmental and physiological challenges. For example, it has been demonstrated that rehousing mice into new cages significantly decreased lactobacilli levels (62), and that chronically sleep depriving rats induced a significant overgrowth of microbiota in the ileum and cecum (22). In addition, individual characteristics of mice, such as age and gender have also been shown to be associated with differential composition of the intestinal microbiota (30).

Fewer studies have focused on the impact of psychological stressors on the microbiota of humans, but an early study in cosmonauts demonstrated that the intestinal microbiota were significantly affected during space flight (47), with other studies suggesting that some of these effects could be due to the stress of confinement (36). To further study the potential impact of psychological stress on the stability of the intestinal microbiota, we assessed the microbiota of young rhesus monkeys that were being separated from their mothers for husbandry purposes (2).

In captive colonies, rhesus monkeys (*Macaca mulatta*) are routinely separated from their mothers at approximately 6 months of age. At this age, the monkeys are no longer nursing and are eating solid foods. Yet, they show a strong physiological and emotional reaction to separation from their mothers (2). This transition from living with the mother to living with other peer monkeys is associated with an increased incidence of diarrhea and loose stools. While much of this can be explained by the impact of the nervous system on gastrointestinal functioning (78), we hypothesized that the stress response to maternal separation could significantly affect microbiota levels in the infants, and thus reduce the barrier effects of the intestinal microflora.

Traditional culture techniques were used to quantify levels of microbiota shed in the stool for one week following maternal separation. These cultures indicated that levels of aerobically grown microbes increased one day after separation, were lower 3 days after separation and returned to baseline levels at the end of the week. When the Gram-negative aerobes and facultative anaerobes were enumerated, a different pattern emerged. In general, levels of Gram-negative microbes steadily increased the week following separation, but the stress-induced differences in the levels of these general groups of microbiota did not reach statistical significance. When a single type of bacteria, mainly the lactobacilli, were quantified, then statistically significant different levels of lactobacilli emerged the week following separation. The levels of lactobacilli shed in the stool was significantly higher one day after separation, in comparison to the pre-separation values. This increase was followed by a significant reduction in the number of lactobacilli shed in the stool 3 days after separation, and a return to baseline levels by the end of one week after maternal separation (2).

Because the lactobacilli are one type of microbiota associated with colonization resistance, we used traditional culture methods to quantify levels of opportunistic pathogens shed in the stool. None of the monkeys in this study were intentionally/experimentally infected with a pathogen; but, the enteric pathogens *Campylobacter jejuni* and *Shigella flexneri* are endemic in many monkey colonies, including the colony at the Harlow Center for Biological Psychology, where this work was performed. And, approximately 42% of the infant monkeys in this study were colonized with at least one of these opportunistic pathogens. To determine if the emergence of the opportunistic pathogens was associated with the stress-induced decrease in lactobacilli, the number of lactobacilli was correlated to the number of opportunistic pathogens shed in the stool. None of the infant monkeys in this study were intentionally/experimentally infected with a pathogen; but, the enteric pathogens *Campylobacter jejuni* and *Shigella flexneri* are endemic in many monkey colonies, including the colony at the Harlow Center for Biological Psychology, where this work was performed. And, approximately 42% of the infant monkeys in this study were colonized with at least one of these opportunistic pathogens. To determine if the emergence of the opportunistic pathogens was associated with the stress-induced decrease in lactobacilli, the number of lactobacilli was correlated to the number of opportunistic pathogens. Overall, there was an inverse correlation between pathogen load and the level of lactobacilli, i.e., animals with higher levels of *C. jejuni* or *S. flexneri* had lower levels of lactobacilli. This association, however, was only marginally significant (*p*=.07) most likely due to the small sample size and the reliance on naturally occurring infections (2). Additional studies are needed to firmly conclude that stress-induced reductions in lactobacilli levels leave the host susceptible to enteric infections.

Stress-induced reductions in lactobacilli have also been found in a study of college students (42). Bacterial levels were assessed during a low stress period (i.e., the first week of the semester) and during a high stress period (i.e., final exam week) to determine whether the stress of taking final exams was sufficient to significantly affect the intestinal microbiota. The exam-period was indeed perceived as stressful for the students; self-reported perceived daily stress and weekly stress were significantly increased as were self-reported cases of

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(2), (54), (30), (47), (78).
gastrointestinal upset. Importantly, the levels of lactobacilli shed in the stool were significantly lower during the examination period, with differences in bacterial levels reaching one half log unit in magnitude (e.g., baseline values of $6 \times 10^7$ CFU/ml vs. $1 \times 10^7$ CFU/ml on Day 5 post-examination). In addition to changing the levels of lactobacilli, exposure to the stressor also led to changes in diet. Specifically, students reported consuming fewer vegetables, but more coffee during the exam period (42). Because the diet is thought to be linked to the microbial composition on the intestines (46), it is possible that stress-induced changes in diet led to the effects on the microbiota. However, given that similar stress-induced effects were found in nonhuman primates fed a standardized laboratory diet (2), it is likely that stress-associated changes in human microbiota reflect an impact of the stressor as well as the effects of diet.

**IMPACT OF A PRENATAL STRESSOR ON THE COLONIZATION OF THE GUT**

Bacteria colonize the gastrointestinal tract of newborns in a sequential pattern that is related to developmental milestones in the infant. This sequential pattern begins at birth when bacteria from the mother’s reproductive tract colonize the sterile newborn. Although the first to colonize, these microbes do not predominate for long and bacteria from the mother’s gastrointestinal tract become the predominant microbes in the infant intestines for the first few days of life. In general, the first microbiota to colonize the intestines are aerobic. As a result, they consume oxygen when they grow and begin to reduce the oxidation-reduction potential in the intestines, thus creating a more favorable environment for the growth of anaerobic species. This results in a pattern marked by high levels of Enterobacteriaceae one day after birth, with anaerobes, such as bifidobacteria, predominating by six days of age. This predominance lasts throughout the period of exclusive breast feeding, with the initiation of weaning triggering a resurgence of aerobic and facultatively anaerobic species, such as *E. coli*, *Streptococci*, and *Clostridia* spp., that are naturally found in the newly consumed foods. Although the levels of these microbes vary greatly during the period of weaning, stable levels of microbes develop as a more consistent diet is maintained, ultimately resulting in the development of a stable ecosystem of commensal bacteria. This ecosystem remains remarkably stable throughout the lifespan, which is a characteristic that is important for maintaining intestinal homeostasis and limiting intestinal infections and cancers (54).

Rhesus monkeys have been widely used to study the effects of gestational stress on offspring development, and it is now known that monkeys born from mothers exposed to stress during gestation have significantly delayed neuromotor development, emotional reactivity to stress, brain monoamine levels, cell density in the brain, and immune reactivity (9, 12–15, 63, 64). Therefore, we used the rhesus monkey model of gestational stress to test the hypothesis that prenatal exposure to a stressor could affect the developmental colonization of the gastrointestinal tract.

Pregnant rhesus monkeys were exposed to an acoustical startle stressor (i.e., 3 random 110 dB beeps over a 10 min period occurring 5 days per week) either early (days 50–92) or late (days 105–147) in the 169 day gestational period. These periods reflect crucial time periods in nervous system and gastrointestinal system development, thus increasing the likelihood that the stressor would disrupt the development of the intestinal microbiota. This stressor was not strong enough to significantly affect the number of miscarriages, gestational length, or birth weight (4), but was sufficient to significantly increase cortisol levels in the pregnant mothers, and to cause significant changes in the development of the intestinal microbiota.

During the first 6 months of life, lactobacilli levels in the monkeys born from mothers exposed to the stressor during gestation were significantly lower than levels found in infants from non-stressed control mothers, with the biggest differences in mean levels found at 2 weeks of age. As successful nursing progressed, bifidobacteria levels began to increase in the infants. And, as with lactobacilli levels, bifidobacteria levels were significantly lower in the intestines of infant monkeys from mothers that were exposed to the acoustical startle stressor during gestation. This effect, however, was only evident in the offspring from mothers exposed to the stressor late in gestation (4). As with the previous studies involving stress in rhesus monkeys, none of these infant monkeys were intentionally/experimentally infected with enteric pathogens. However, approximately 43% of the infants from mothers stressed early in gestation and 12% of the infants from mothers stressed late in gestation became subclinically colonized with *S. flexneri*, an endemic pathogen in the monkey colony. Importantly, *S. flexneri* were not detected in any of the infants born from the non-stressed control condition (4) suggesting that prenatal stress, particularly early in gestation, disrupted the development of natural resistance to the enteric pathogen, *S. flexneri*. 
STRESS AND INTESTINAL MICROBIOTA

IMPACT OF RODENT STRESSORS ON THE INTESTINAL MICROBIOTA

Rodents are used for many biomedical studies, and have proven to be useful in studies on gastrointestinal disorders and infection. Moreover, there is growing use of rodents to study intestinal microbiota, and many rodent stressors are well defined in terms of the behavioral and endocrine stress responses and the resultant impact on the immune system and other organ systems. Prolonged restraint is one stressor that is widely used in both rats and mice and has been shown to have a number of effects on immunity as well as gastrointestinal functioning (7,70).

Therefore, we used prolonged restraint to determine the impact of the stressor on the intestinal microbiota. In our model, male CD-1 mice were placed into a 50 ml conical centrifuge tube with holes for ventilation for 15 hr during their active cycle. After the 15 hr period, the mice were removed from the tube and left undisturbed for 9 hr. The stressor was then repeated after this 9 hr rest period. Therefore, each restrained animal was in the restraining tube for 15 hr per day for up to 7 consecutive days. After 1, 3, 5, or 7 days of repeated restraint, fecal samples were collected to enumerate the microbiota using differential agars to grow total (i.e., both Gram-positive and Gram-negative) aerobes and facultative anaerobes as well as just the Gram-negative aerobes.

Exposure to repeated cycles of prolonged restraint results in significantly elevated circulating levels of the adrenal hormone corticosterone, indicating that the paradigm is sufficient to induce a physiological stress response [see for example (57)]. However, unlike stressors in nonhuman primates, restraint stress resulted in higher levels of bacteria being shed from the intestines as evidenced by a significant increase in total aerobic and facultatively anaerobic bacteria \[F(4, 35) = 3.99, p<.05\] (Fig. 1A), and Gram-negative aerobic and facultatively anaerobic bacteria \[F(4, 35) = 1.15, p<.05\] (Fig. 1B) over the 7 day stress period \((p<.05)\). Interestingly, a single exposure of 15 hr of restraint was sufficient to increase the mean level of total as well as Gram-negative aerobes and facultative anaerobes, but statistically significant increases in the levels of the Gram-negative microbes were not reached until 3 cycles of repeated restraint \((p<.05)\). The overgrowth in Gram-negative microbes is particularly striking, since small intestinal bacterial overgrowth is one factor that has been shown to lead to bacterial translocation from the gut (66). This is consistent with a previous study from our group showing that exposure to prolonged restraint stress resulted in bacterial translocation as indicated by a significant increase in the prevalence of microbes cultured from the mesenteric lymph nodes (3).

The effects of prolonged restraint stress on individual species of microbiota, particularly the anaerobic species, has not yet been studied. However, we have been using molecular sequencing techniques, i.e., 454 pyrosequencing, to determine the effects of this stressor on the composition of the microbiota. It is hoped that the use of this methodology will confirm the stress-induced effects on lactobacilli and bifidobacteria, and also lead to the identification of genera or species of microbiota that

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Fig. 1. Mice were exposed to repeated cycles of 15 hr of restraint for up to 7 days. The day after restraint, stool samples were collected from the mice \((n=3–6\) per group per day) and the levels of total aerobic and facultatively anaerobic bacteria were determined by growth on brain heart infusion agar incubated aerobically for 18 hr at 37°C (Fig. 1A). The levels of Gram-negative aerobes and facultative anaerobes in the stool samples were determined by growth on the differential agar eosin methylene blue incubated aerobically for 18 hr at 37°C (Fig. 1B). Because food and water is not available while the mice are in the restraint tubes, FWD Control mice were deprived of food and water during the same time period that the Restrained mice were in the restraining tubes. * \(p<.05\) vs. FWD Control on individual culture days.
are important for maintaining the balance between health and disease.

**PSYCHOLOGICAL STRESS AND THE GI TRACT: PROPOSED MECHANISMS OF STRESS-INDUCED ALTERATIONS IN MICROBIOTA**

The complete set of factors that regulate microbial populations in the gastrointestinal tract are not well understood. It is known, however, that several physiological properties, such as gastric acid secretion, gastrointestinal motility, mucous secretion, bile secretion, and secretory immune factors can influence the types and levels of bacteria that can reside as part of the microbiota. For example, it is well known that ingested bacteria must survive the low pH of the stomach prior to being able to colonize lower sections of the GI tract. In support of this notion, it is recognized that reduced production of gastric acid (which can occur during hypochlorhydria) results in overgrowth of bacteria in the GI tract (21). Thus, secretion of gastric acid helps to reduce microbial levels in the GI tract, and also gives a selective advantage to microbes that can survive in the harsh gastric environment.

Interestingly, emotional states and psychological stress are well known to affect the secretion of gastric acid. This was observed over 170 years ago when it was found that fearful responses in a patient with gastric fistula resulted in significantly reduced or abolished gastric acid secretion (5). Modern experimental data has confirmed this observation, and it is now known that secretion of gastric acid can be suppressed by experimental stressors in humans, such as the cold pressure task and mental arithmetic (1, 37). Similar findings have been observed in experimental animals, where different stressors have been shown to have differential effects on acid secretion, with restraint stress reported to significantly increase or decrease gastric acidity depending upon body temperature (44, 53). Recent work in the field of neurogastroenterology has demonstrated that these differences are due to different levels of activation of the sympathetic and parasympathetic nervous systems; activating the SNS suppresses whereas activating the PNS enhances acid secretion (79).

Members of the genera *Lactobacillus* and *Bifidobacterium* (as well as other members of the microbiota) are acid tolerant and can remain viable in low pH conditions (21). Therefore, one hypothesis is that during exposure to a stressor, gastric acid levels are decreased thus causing lactobacilli and bifidobacteria to loose their ecological advantage over other bacterial species. Research is needed to determine whether GI acidity plays a role in stress-induced alterations of microflora and to test alternative hypotheses of how stress affects the intestinal microbiota.

Gastric acid secretion is not the only GI physiological process affected by psychological stressors. For example, GI motility, which has long been thought to influence microbial populations in the GI tract, can be either slowed or enhanced by exposure to stressors depending upon the type of stressor and the region of the gastrointestinal system analyzed (69, 70). Psychological stress is also well known to influence the immune response, and the importance of immune factors in influencing indigenous microbial populations is becoming more recognized. Mice unable to produce secretory immunoglobulin A (sIgA) have significantly increased populations of anaerobic microbiota in their small intestine demonstrating the important role of sIgA in helping to control microbiota populations (23). In addition to sIgA, antimicrobial peptides, such as the defensins, have been suggested to modify the types and numbers of bacteria colonizing the gastrointestinal tract (61). Because both sIgA and the defensins can be influenced by exposure to stress (29, 39, 43, 59), a challenge for future research is to determine whether stress-induced changes to these immune molecules affects microbial populations in the intestines.

An equally valid hypothesis is that the intestinal microbiota were directly affected by stress-induced increases in intestinal hormones, such as norepinephrine. The growth of many types of bacteria, including infectious organisms as well as members of the indigenous microbiota, have been shown to be significantly enhanced upon culture with NE (28). However, despite the many studies indicating that bacterial growth can be affected by neuroendocrine hormones in culture, proving that these interactions occur *in vivo* has been challenging. Neuroendocrine-bacterial interactions, however, are likely to occur *in vivo* when hormone levels are high enough. For example, in our previous study where sympathetic nerve terminals were lysed with the drug 6-hydroxydopamine (6-OHDA), which results in a large outpouring of the sympathetic neurotransmitter NE (18, 60), bacterial levels in the cecums of mice were found to be significantly increased (50). Because the majority of most of these bacteria were identified as *E. coli* (50), and because the growth of commensal *E. coli* is strongly affected by exposure to NE (28), the data suggest that overgrowth of *E. coli* was the result of direct enhancement by NE. A remaining challenge for this field is to determine the mechanisms through which psychological stressors can affect the
microbiota. Recent developments of molecular techniques to identify microbes, advances in biofilm research, and new discoveries within the field of neurogastroenterology make it an ideal time to systematically study how the stress response impacts the intestinal microbiota.

CONCLUSIONS

The past ten years have seen an expansion in our understanding of the important roles that the intestinal microbiota play in maintaining human health. While their importance as a barrier to enteric pathogens has been recognized for many years, it has only been recently that the importance of the microbiota for more complex disorders has become realized. It is now thought that the microbiota are involved with obesity and diabetes (76), as well as with IBS and IBD (58). Although the link to IBD has been well supported, newer studies are now finding abnormal microbial populations in IBS patients as well (40). However, it has been difficult to link IBS to a specific microbial species, in part due to the wide range of techniques, differing patient groups, and the complexity of the GI microbiota alone [see (58) for review]. However, an association between IBS and lower luminal levels of lactobacilli and bifidobacteria has been consistently found particularly in patients with diarrhea-associated inflammatory bowel disease (40). The influence of the intestinal microbiota, however, may not fit a single microbial species. And, it is possible that a more global alteration in the ecosystem leads to IBS due to a dysregulation of intestinal pH, immune homeostasis, and/or metabolism of hormones [see (55) for review].

Our results, and the results of others over the past 50 years, strongly indicate that exposure to psychological and environmental stressors significantly alters microbial populations in the intestines. This has been evident in mice, rats, nonhuman primates, and humans exposed to a variety of different stressors ranging from cold exposure to school examinations. However, our understanding of the nature of the relationship between the stress response and stress-induced changes in the intestinal microbiota is only in its infancy. This is in part due to the reliance on culture-dependent methodologies that preclude a simultaneous analysis of many different species of microbes. As the use of molecular, culture-independent methodologies becomes more widespread in studies involving the use of a stressor, it will become clear which species of microbes are most influenced by the stress response. Moreover, the use of different types of stressors that in turn result in different types of stress responses will help to elucidate the mechanisms through which stress affects the microbiota.

Perhaps the most important challenge for this field, however, is determining the health importance of stress-induced alterations in the intestinal microbiota. This link should not seem unreasonable, however, given that many diseases and conditions that have been linked to the intestinal microbiota are also known to be exacerbated during periods of psychological stress. For example, IBS has long been thought to be worsened during periods of psychological stress, to the extent that early descriptions of the disease described it as psychosomatic (35, 77). While stress may have a variety of effects on the gastrointestinal tract that are important to the manifestation of gastrointestinal diseases, our results suggest that stress-induced alterations in the microbiota are an additional factor in these complex diseases. Future studies, utilizing well characterized animal models in addition to well controlled translational studies will help to elucidate the impact of stress-induced changes in the microbiota on the health of the host.

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