Special Article

Early-life enteric infections: relation between chronic systemic inflammation and poor cognition in children

Reinaldo B. Oriá, Laura E. Murray-Kolb, Rebecca J. Scharf, Laura L. Pendergast, Dennis R. Lang, Glynis L. Kolling, and Richard L. Guerrant

The intestinal microbiota undergoes active remodeling in the first 6 to 18 months of life, during which time the characteristics of the adult microbiota are developed. This process is strongly influenced by the early diet and enteric pathogens. Enteric infections and malnutrition early in life may favor microbiota dysbiosis and small intestinal bacterial overgrowth, resulting in intestinal barrier dysfunction and translocation of intestinal bacterial products, ultimately leading to low-grade, chronic, subclinical systemic inflammation. The leaky gut–derived low-grade systemic inflammation may have profound consequences on the gut–liver–brain axis, compromising normal growth, metabolism, and cognitive development. This review examines recent data suggesting that early-life enteric infections that lead to intestinal barrier disruption may shift the intestinal microbiota toward chronic systemic inflammation and subsequent impaired cognitive development.

INTRODUCTION

Despite technological advances of the 21st century, diarrheal diseases still account for a staggering death toll worldwide in children under 5 years of age, largely owing to poverty combined with deplorable yet potentially remediable poor sanitation and hygiene. Most deaths occur in developing countries, with the largest numbers recorded in sub-Saharan Africa and Asia, where poverty is the greatest. Over the past 2 decades, mortality in children under 5 years of age has decreased substantially, but reductions have been variable in resource-limited regions.[1,2] Improvements in global health initiatives, such as oral rehydration therapy, have contributed to declines in mortality due to diarrheal disease, yet 1.3 million people still die every year across all ages.[3] In 2011, 700 000 children under 5 years of age died because of sequelae of diarrheal illness, with a high proportion of deaths occurring in the first 2 years of life.[2] However, a considerable proportion of the enteric infection burden is subclinical and may be present in a chronic or prolonged state. Since this type of infection does not necessarily lead to hospitalization, it is a silent and neglected health issue that is difficult to track and likely to be largely unrecognized by official health statistics.

Recently, much effort has gone into understanding how prolonged or repeated enteric infections (with or without diarrhea) are associated with an unhealthy gut environment that may lead to metabolic and even epigenetic consequences, with lasting negative effects on growth, cognition, and educational achievement that can reduce the likelihood of an individual reaching his or her full potential.[4–6] Prolonged or repeated enteric

Affiliation: *R.B. Oriá* is with the Laboratory of Tissue Healing, Ontogeny and Nutrition, Institute of Biomedicine and Department of Morphology, Faculty of Medicine, Federal University of Ceará, Ceará, Fortaleza, Brazil. *L.E. Murray-Kolb* is with The Pennsylvania State University, University Park, Pennsylvania, USA. *R.J. Scharf, G. Kolling,* and *R.L. Guerrant* are with the Center for Global Health, Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, Virginia, USA. *L.L. Pendergast* is with the School Psychology Program, Temple University, Philadelphia, Pennsylvania, USA. *D.R. Lang* is with the Foundation for the National Institutes of Health, Bethesda, Maryland, USA.

Correspondence: *R.B Oriá*, Institute of Biomedicine and Department of Morphology, Faculty of Medicine, Federal University of Ceará, Fortaleza-CE, Brazil. Email: rbo5u@virginia.edu. Phone: +55-8533668239.

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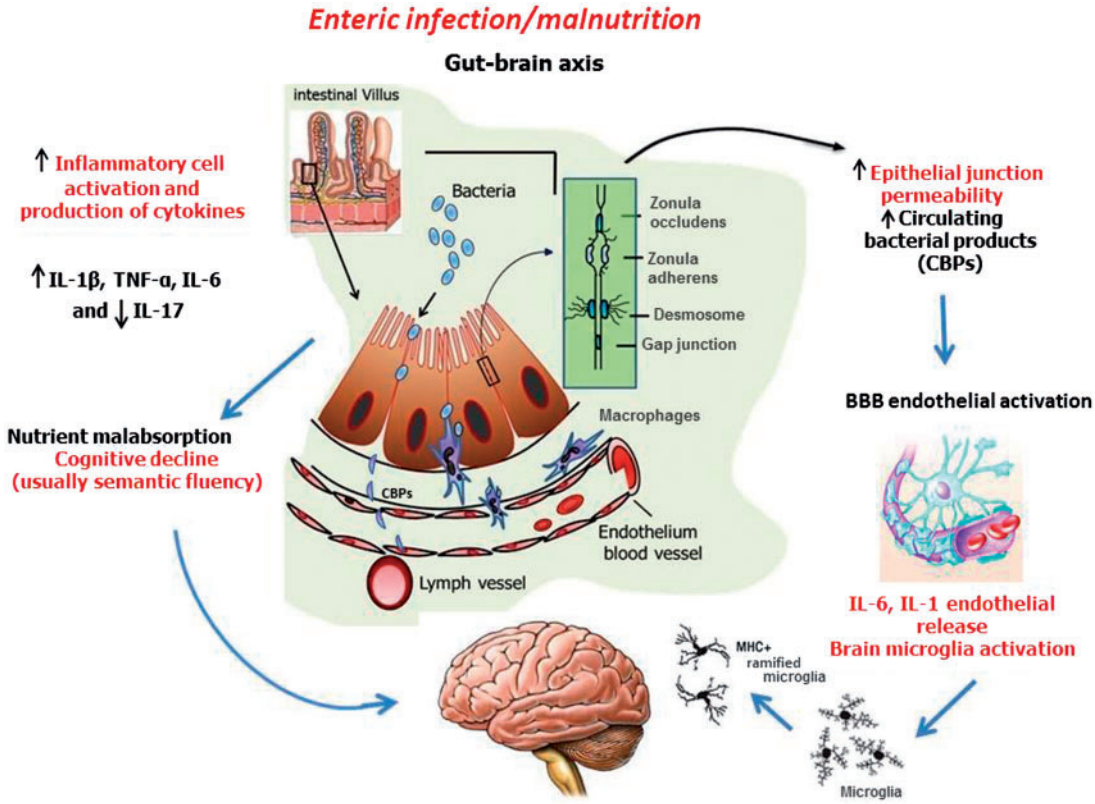

Figure 1 **Proposed model of how a prolonged state of enteric infection/malnutrition causes systemic inflammation, thereby affecting the gut–brain axis.** Malnutrition and repeated enteric infection in the first 2 years of life may cause intestinal barrier leakage. Intestinal dysbiosis may facilitate luminal-to-blood pathogenic bacterial translocation and, consequently, low-grade systemic inflammation. Circulating bacterial products may activate the endothelial cells, which form the blood–brain barrier, to release proinflammatory cytokines to prime and activate microglial cells. An early-in-life subclinical neuroinflammatory state may affect cognitive development in children. *Abbreviations and symbols*: ↑, increased; ↓, decreased; BBB, blood–brain barrier; CBPs, circulating blood products; IL, interleukin; MHC, major histocompatibility complex; TNF-α, tumor necrosis factor-α.

infections have long-lasting consequences, since the effects may extend throughout a lifetime and may even be multigenerational, leading to an unresolved vicious cycle of poverty, low education, and costly poor health and well-being of individuals and society.[7]

Although chronic low-grade intestinal inflammation has been increasingly recognized as a factor contributing to poor intestinal absorption of nutrients,[8] the underlying mechanisms remain poorly understood. Recently, it has been shown that genetically engineered mice with toll-like receptor 5 deficiency only in enterocytes demonstrate low-grade inflammation and develop metabolic syndrome. These effects were associated with changes in the intestinal microbiota and levels of bacterial lipopolysaccharide (LPS) and flagellin, since antibiotic treatment reduced these effects.[9]

In this review, novel findings pointing to increasing evidence of the often-hidden costs of the "impoverished gut" are examined, shedding light on how enteric pathogens (often coupled with inadequate diets) can shift the balance of the intestinal microbiota toward inflammation, malabsorption, and potential low-grade liver and brain inflammation and have significant consequences for metabolism and cognitive development in children afflicted by enteric diseases early in life. Figure 1 shows a proposed model of how prolonged (even if subclinical) intestinal barrier dysfunction can lead to a status of chronic local and systemic inflammation, with profound effects on brain development.

Environmental enteropathy and the intestinal microbiota

Intestinal microbiota. The intestinal microbiota, approximately 100 trillion microbial cells, is composed of a remarkable range of bacterial (and other microbial) taxa that outnumber human body cells. The most abundant bacterial species are members of the phyla Firmicutes and Bacteroidetes, with smaller numbers being representative of the phyla Proteobacteria, Fusobacteria, Cyanobacteria, Verrucomicrobia, and Actinobacteria, among others.[10,11] The gut microbiota is composed mainly of anaerobes, which outnumber facultative anaerobes and aerobic bacteria by

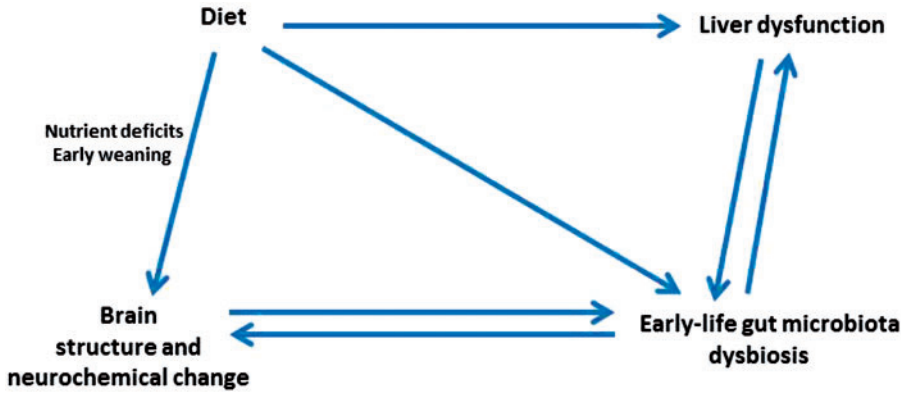

Figure 2 **Dynamic interactions between diet, intestinal microbiota, brain, and liver.**

approximately 2 to 3 orders of magnitude.[12] The intestinal microbiota exists in a symbiotic relationship with the host that is reflective or a result of interactions with an intact intestinal mucosal barrier and an effective mucosal immune system.[13] Changes in both bacterial abundance and species diversity within the gastrointestinal tract are locale dependent; specifically, a dynamic, diet-luminal-driven microbiota, which may alter intestinal absorptive capacity, is present, especially in the distal small bowel.[14]

Dietary factors, intestinal immunity, and the microbiota. The intestinal microbiota is in a state of flux during the first 3 years of life before stabilizing[15] and is affected by enteric infections and changes in diet that shape the mucosal immune system and influence nutrient absorption, thus having potential long-term consequences.[16] Dietary factors (such as nutrient composition and change in diets) are key determinants of the composition of the microbiota and may influence the numbers of intestinal bacteria.[17] Figure 2 shows the dynamic interactions between dietary factors and the intestinal microbiota that may profoundly affect liver and brain functions early in life. These interactions have resurged as an intense area of research.[18–21] Potentially harmful bacteria proliferate under certain conditions in the intestinal lumen and may show increased translocation patterns, disrupting the balance of homeostatic translocation of commensal bacteria needed to induce mucosal production of immunoglobulin A.[22] Proliferation of pathogenic bacteria may be amplified when intestinal motility is inadequate. If this inadequate motility is chronic and low grade, it may not trigger a diarrheal response, which might otherwise lead to washout of luminal bacterial overgrowth. There is a close relationship between the intestinal microbiota and mucosal immunity. Intestinal bacteria have a role in human intestinal B-cell function[23] and the formation and activity of intestinal T_H17 effector T cells.[24] They also suppress production of regulatory T cells. Commensal bacteria are important to control regulatory T cell populations in the small intestine.[25] Certain components of the gut microbiota are critical to the induction of regulatory T cells.[26,27] In a recent study, mice receiving orally administered microbiota enriched with regulatory T-cell–inducing species showed improved outcomes in models of allergic diarrhea and experimental colitis after 3 weeks of treatment.[28]

In the intestinal luminal environment, bacterial symbionts such as *Bacteroides* may control the population of harmful enteric pathogens such as *Salmonella* and *Shigella* species, which have a more limited capacity to produce saccharolytic enzymes.[29] Consequently, these pathogens are poorly adapted to compete with symbionts for nutrients from the host diet, restricting their luminal colonization.[30] In addition, symbiotic bacteria may reduce the virulence of enteric pathogens by stimulating epithelial Toll-like receptors. This stimulation triggers the expression of a variety of antimicrobial proteins that might play a role in limiting the penetration of enteric pathogens into the epithelial barrier.[31]

As enteric pathogens colonize the gut, they may induce intestinal inflammation and excess free radicals. Infection with *Salmonella typhimurium* induces inflammation and production of reactive oxygen species via NADPH (nicotinamide adenine dinucleotide phosphate) oxidase activity during respiratory burst of phagocytes.[32] Reactive oxygen species oxidize thiosulfate (which alone may be relatively innocuous), which is produced by the epithelium, resulting in the production of tetrathionate. This compound facilitates respiration by *Salmonella*, providing a growth advantage to this pathogen.[33] In addition, overt inflammation in the intestinal milieu may cause a state of disequilibrium in the microbiome that favors overgrowth of some pathogenic species, including certain *Escherichia coli* pathotypes.[34]

Helminths and intestinal microbiota. A small number of studies have investigated the impact of helminthic

infections on the intestinal microbiota and inflammation.[35,36] A recent study evaluated microbial communities after nematode infection and showed significantly higher levels of cecal γ-Proteobacteria/Enterobacteriaceae and *Bacteroides/Prevotella* organisms than in uninfected controls, which may constitute an anti-inflammatory factor in inflammatory bowel disease.[37] Interestingly, Cantacessi et al[35]. did not find significant changes in the intestinal microbiota communities after *Necator americanus* infection in healthy volunteers, but they did not evaluate subjects living in areas where enteric infections were endemic. One recent study assessing juvenile rhesus macaques with idiopathic chronic diarrhea did find amelioration of the intestinal barrier function and reductions in T_H1 inflammatory gene expression (and activation of mucosal T_H2 response) following infection with whipworm *Trichuris trichiura*. These alterations in intestinal inflammation were accompanied by changes in the microbial communities associated with the intestinal mucosa.[38] Nevertheless, it is not known how different classes of helminths and helminthic burden interact with other intestinal comorbid conditions to influence intestinal inflammatory responses.

Small intestinal bacterial overgrowth. Small intestinal bacterial overgrowth is considered a result of intestinal dilation and stasis, which together promote excess bacterial proliferation and inflammation.[39,40] It may occur when the small intestinal microbiota is altered[41] and is likely affected by diet (e.g., high-carbohydrate diets with resultant luminal distension caused by impairment of digestion and absorption and changes to the composition of the luminal contents). Small intestinal bacterial overgrowth may lead to more inflammation and impaired nutrient absorption. In addition, it may lead to malabsorption of dietary fat, with important health consequences for children with enteric infections, when energy intake is important for catch-up growth.[42,43] Small intestinal bacterial overgrowth (detected by increased intestinal hydrogen and methane concentrations in breath) has been identified in Brazilian children with poor nutritional status living in slums.[44,45]

Small intestinal barrier dysfunction may lead to small intestinal bacterial overgrowth,[45] thereby causing dysbiosis in the proximal intestine. Small intestinal bacterial overgrowth has been correlated with liver steatosis (nonalcoholic fatty liver disease),[46] an escalating health condition worldwide. In addition, reduced liver function leads to lower levels of potentially bacteriostatic bile acids (as well as small intestinal bacterial overgrowth by inducing bile acid deconjugation and fatty acid diarrhea[47]) that reach the small intestine through the enterohepatic circulation. Corroborating data demonstrate that administration of conjugated bile acid to rats with cirrhosis improves bile secretion and reduces intestinal bacterial overgrowth and translocation.[48] There is little information available regarding bile acid and parasite growth; however, some findings suggest that bile acids may regulate *Cryptosporidium parvum*[49] and *Giardia lamblia*[50] excystation and invasiveness.

Commensal intestinal bacteria are considered key to maintain the intestinal epithelial barrier function. Studies using probiotics have documented improved expression of the zonula occludens-1 protein in a model of dextran sodium sulfate-induced colitis in BALB/c mice.[51,52] The dysbiosis or disruption of resident microbiota induced by enteric pathogens may affect the regulatory benefits of commensal bacterial to the intestinal epithelial barrier.

Environmental enteropathy and liver function

Intestinal bacterial translocation and liver clearance of LPS. Environmental enteropathy increases the intestinal proinflammatory state during chronic intestinal epithelial breakdown, which in turn is associated with overgrowth and translocation of small intestinal bacteria. Key proinflammatory cytokines can trigger the secretion of acute-phase liver proteins and enhance both the production of B-cell antibodies and the proliferation of T cells. If malnutrition occurs simultaneously, the latter effect is more likely compromised.[53] In addition, the cytokine-mediated inflammatory response can further impair the intestinal barrier function, causing even more bacterial translocation. In addition to causing low-grade systemic inflammation, translocated bacterial products can enter the liver from the small and large bowel via the portal circulation and reach the space of Disse near the hepatocytes. Kupffer cells are the main liver cells responsible for phagocytosis of bacteria and therefore respond to any liver insult. Any impairment in liver function may compromise the clearance of LPS. Increased intestinal interferon-γ levels have been associated with impaired intestinal cell monolayers in vitro, along with depletion of tight-junction proteins.[54] Interestingly, in patients with Crohn's disease who show subclinical intestinal inflammation, a marked positive correlation between fecal lactoferrin, serum C-reactive protein (a liver acute-phase response), and interleukin 6 was identified[55] following bowel resection. Unpublished data (A.A.M. Lima, M.D. DeBoer, and R.L. Guerrant, et al.) support this association between intestinal inflammatory markers and systemic inflammation, showing a positive correlation between serum C-reactive protein and fecal myeloperoxidase (a marker of intestinal inflammation) in Brazilian children attending a nutrition clinic in an area of endemic malnutrition and enteric diseases.

Environmental enteropathy–driven systemic inflammation, disrupted iron metabolism, and brain myelination. Another important aspect of the leaky gut–induced systemic inflammation is related to iron metabolism. It is noteworthy that systemic inflammation may directly affect intestinal absorption of iron by sequestrating iron within intestinal enterocytes.[56] One mechanism is through an increase in the hepatic hormone hepcidin, which binds to ferroportin, causing it to be internalized and, hence, preventing the efflux of iron from the enterocyte.[38] Inflammation can also upregulate hepcidin synthesis by various brain cells (astrocytes, microglia, and neurons), which results in accumulation of intracellular iron and lack of iron availability for important developmental processes.[57] These changes in iron metabolism may be detrimental to cognitive development, as oligodendrocytes require iron for myelin synthesis, a process accomplished by the release of iron from neuronal iron stores during myelination. Iron is also required for monoamine synthesis, neuronal metabolic activity, and the proper development of neuronal morphology.[58,59] All of these processes are compromised when increased hepcidin levels reduce neuronal ferroportin activity.[60] This is problematic, especially during the first 2 years of life, when myelination and dendritic arborization are occurring rapidly. In addition, parasitism early in life (especially due to ancylostomiasis) would potentially amplify this deleterious effect by exploiting the host.[61] In mouse studies, the intestinal microbiota and intestinal inflammation were able to modulate liver hepcidin expression.[62] Monocytes and macrophages also express hepcidin, which is regulated primarily by inflammatory mediators and infectious agents.[63]

Given the importance of iron for proper brain development, iron supplementation may be prudent in certain populations, especially in those with a high prevalence of deficiency. However, areas of the world where prevalence of iron deficiency is high are often areas where susceptibility to enteric infections is also high, leading to an inflammatory state within affected individuals. As noted above, this inflammation is likely to upregulate the production of hepcidin, which prevents the release of iron from enterocytes, thereby greatly reducing the efficacy of iron supplementation. In addition, there is concern that iron supplementation in such individuals may trigger a more proinflammatory state in the intestinal tract, as iron supplementation may favor bacterial proliferation.[43] Iron supplementation may not be adequate if the causal mechanism is intestinal bacterial dysbiosis, since iron supplementation may even favor bacterial proliferation and therefore sustain a more proinflammatory state in the intestinal tract.[64]

Chronic systemic inflammation (even if low grade) may alter brain development, which may result in lasting neurological deficits without leading to conspicuous brain lesions. In addition, neonatal systemic inflammation disrupts white-matter programming, leading to impairments in oligodendrocyte and axon maturation, likely more pronounced during a time of increased vulnerability.[65] In addition, gut microbiota may influence the deleterious effects of chronic exposure to heavy metals on the brain.[66]

Iron supplementation and cognition

Multiple studies have assessed the relation of iron supplementation with cognitive development in children under 5 years of age.[67] Studies conducted in infants have consistently reported findings of lower mental development test scores,[68] lower motor test scores,[68,69] and differences in social-emotional behavior[70] in those who are iron-deficient anemic vs those who are iron sufficient. However, these same studies report persistent cognitive differences in the groups, even after iron treatment. These findings are generally interpreted as the iron deficiency having occurred during a critical period of development, such that the negative effects are not reversible with supplementation during this age. When examining the studies of iron supplementation and cognitive development in toddlers and preschool-aged children, there are consistent findings of lower scores on learning tasks and language and motor development in children who are iron-deficient anemic vs those who are iron sufficient. These individual studies show remarkable agreement about supplementation reversing these negative effects. However, meta-analyses of such studies have not been consistent in finding support for supplementation.[71] Although many of these studies conducted in children under 5 years of age were carried out in populations susceptible to repeated enteric infections, none of them examined the relation between enteric infection, the intestinal microbiota, the iron status of the individual, and cognitive development.

Environmental enteropathy and genetic predisposition

APOE4 as a possible protective factor. Some of the immune system responses to enteric pathogens may be strongly influenced by the host's genetic background.[72,73] One interesting gene is the apolipoprotein E4 gene (*APOE4*), a gene related to cholesterol transport and metabolism and increasingly recognized to have important immune-inflammatory roles.[74]

Children from a Brazilian shantytown bearing *APOE4* and raised in areas where diarrhea and enteric infections are endemic have been found to have improved cognitive development when compared with non-*APOE4* neighbors[75,76]; however, this was observed only in children who experienced heavy diarrhea burdens. In addition, one study documented a beneficial effect of *APOE4* in reducing circulating C-reactive proteins in individuals exposed to heavy infection loads in an indigenous Bolivian population.[77] Interestingly, in a recent hospital-based case–control study, individuals who harbored *APOE4* had less nonalcoholic fatty liver disease.[78] Undernourished *APOE4*-targeted replacement mice show better adaptation against experimental *Cryptosporidium parvum* infection than controls.[79] Cryptosporidiosis has been found to be a strong factor to modulate the intestinal microbiota in immunosuppressed mice.[80] More studies are needed to appreciate the effects of *APOE4* in children with environmental enteropathy and the relationship with gut and liver function. Although *APOE4* is a compelling target gene for understanding causality, the multifactorial effects of environmental enteropathy should highlight an array of genes, especially since multiple genes are likely to have lasting effects on cognition in children. Metagenomics of the early intestinal microbiota in light of environment exposures and host genetics is key for deciphering novel determinants of environmental enteropathy, especially when coupled with the study of innate immune-related genes.[81]

Microbial ecology and brain development in the first 2 years of life

Timeline of intestinal colonization from 0 to 24 months. The construction of the intestinal microbiota starts after birth[82] and continues to be actively remodeled over the first 3 years of life, reaching a maturation status similar to that of an adult.[83,84] A number of factors play a role in shaping the intestinal microbiota (and other host-associated microbial communities), including mode of delivery, environmental exposures, and diet. Vaginal or cesarean delivery of infants impacts colonization of the intestinal tract by representative species from vaginal or skin communities, respectively. Intestinal colonization in the postnatal period is further shaped by the diet and is reflective of the metabolic capacity,[85,86] a trend that continues into childhood and beyond.[87,88] A small portion of microbes from other sources (e.g., environment, animals, water) are classified as pathogens, which introduce additional variability to the intestinal microbiota.

Enteric pathogens: prevalence from 0 to 24 months and links to brain function. Increased pathogen exposure during the first 2 years of life is coincident with exposure to different reservoirs that include food,[89,90] hygiene-related behaviors,[91–93] and contaminated water sources.[94,95] Global studies designed to measure the burden of diarrhea around the world highlight the prevalence of numerous pathogens associated with pediatric diarrhea.[96–98] Pathogens with the highest prevalence across study sites include *Campylobacter* spp, rotavirus, enterotoxigenic *E. coli*, *Cryptosporidium* spp, and *Shigella* spp; several other enteric pathogens were identified in the studies but were locale dependent.[99,100] In addition to the pathogen burden during diarrhea, the intestinal microbiota is also affected by lower bacterial diversity and higher levels of facultative anaerobes.[101–103]

Unearthing the impact of enteric disease on brain development and function in children is an underappreciated area[11]; nevertheless, research efforts are under way to understand the breadth of the relationship between enteric infection and brain function. Among the studies of predominant diarrheal pathogens in children, at least two reported associations of *Cryptosporidium* or *Giardia* infection and diarrhea in early life with cognitive function in later life.[104,105] Rotavirus infection can potentially affect the central nervous system in pediatric patients,[106] although the long-term impacts of this infection are unknown. In rodent models, systemic infection or LPS exposure during the postnatal period affects brain development and function in adulthood,[107,108] signifying both pathogens and commensal organisms as contributing factors.

Additional factors that likely play a role in enteric infections and brain development/function during the first 2 years of life include hydration status and diet. Childhood diarrhea inevitably alters host hydration status, resulting in lower diversity of the intestinal microbiota.[103] Dehydration is known to affect cognitive function in children and young adults, although information about its impact in early childhood is limited.[109]

Diet during the first 2 years of life. As previously mentioned, microbial shifts in the intestinal community are coincident with the diet[110]; furthermore, remodeling of the intestinal microbial community during the introduction of solid food appears to be dependent on whether infants were breastfed.[86] The quality and type of foods within a given diet influence the composition of the microbiota and, ultimately, host health (reviewed by Mitsuoka[111] and Subramanian et al[112].). A recent study found that malnutrition is associated with intestinal microbiota immaturity, which was only partially ameliorated by nutritional intervention in Bangladeshi children. The intestinal microbiota immaturity was measured by two indices, named the "relative microbiota maturity index" and the "microbiota-for-age *Z*-score," the latter

calculated on the basis of healthy children of similar chronologic age.[113] Future research would potentially address whether intestinal microbiota immaturity is closely related to early-life enteric pathogen burden and whether it impacts developmental cognitive function.

One aspect of host health already mentioned is brain function. In the absence of infection, protein-energy malnutrition has an adverse effect on behavior compared with nutrient sufficiency in rats.[114,115] Additional studies show that behaviors of undernourished dams and their offspring are negatively impacted,[115,116] linking diet and microbiota (by proxy) to brain function. Metabolism of food by the host (and perhaps by the mother) has the potential to affect host genetics.

Brain development, metabolites, and the microbiota from birth to age 2. The number of articles establishing links between the intestinal microbiota, the gut, and the brain has increased exponentially over the last decade, beginning with topics in the area of microbial endocrinology.[117–119] The majority of studies utilize germ-free mouse systems to examine the impact of microbial colonization on brain-related functions, but these models are typically carried out in mature mice. Nevertheless, data from human and mouse studies can be combined to start building hypotheses. For example, a recent study measured important brain metabolites from birth to adolescence,[120] some of which are exclusively part of human metabolism (e.g., *N*-acetylaspartate) and others that are acquired through diet and/or, potentially, the intestinal microbiota (e.g., myo-inositol). In humans, levels of myo-inositol decrease over the first 5 years of life, coincident with colonization and stabilization of the intestinal microbiota.[83,84,120] To extend this further, a recent study conducted transcriptional and metabolomics analyses during mouse conventionalization and found positive correlations between measured metabolites and related host metabolic genes. Furthermore, the data suggest that myo-inositol levels decrease upon conventionalization, suggesting a role for the microbiota in regulating these levels.[121] As more large-scale datasets are made available to the scientific community, additional interactions between the microbiota and host can be unraveled.

Potential effects of early-life intestinal microbiota on the brain. The first 2 years of extrauterine life is a key time window for human brain development, with profound remodeling of synaptic circuitry, active processes of synaptogenesis and synaptic pruning, and myelination[122] all occurring within the same time period that dietary transition from breastfeeding to solid foods takes place. Often, this transition is accompanied by the introduction of contaminants and pathogens in areas of poor environmental hygiene, facilitating the onset of early enteric infections.[123,124]

In addition, the maturation of the blood–brain barrier early in life has been recognized in mouse experiments to be regulated by the intestinal microbiota, which can affect blood–brain barrier tight-junction proteins, regardless of brain vascular density, having effects that last into adulthood.[125] It is unclear how enteric infections early in life may affect the blood–brain barrier function by disrupting the normal intestinal microbiota in humans. Furthermore, early-life gut microbial populations may affect brain neurotransmission, increasing the risk for developmental neuropsychiatric disorders, such as autism and schizophrenia.[126,127] It is noteworthy that luminal intestinal microbiota have been found to induce serotonin biosynthesis by stimulating enterochromaffin cells, which increase the supply of serotonin to circulating platelets.[118]

It is not known whether increased production of intestinal luminal serotonin is a mechanism that can potentially trigger later neuropsychiatric disorders. Recent studies have documented that intestinal microbiota produce different neurotransmitters,[119,128,129] but it is unknown whether gut-derived neurotransmitters affect brain functions, especially during early brain development, and whether enteric pathogens could disturb these processes. Interestingly, Diaz Heijtz et al.,[130] by conventionalizing germ-free mice early in life with the gut microbiota from specific-pathogen-free mice and testing anxiety and motor behaviors into adulthood, speculate that those behaviors may be programmed soon after birth, when the newborn mice are exposed to gut microbiota, since gut microbes may modulate levels of important neurotransmitters and their metabolites in the striatum in the adult mice.

An interesting study from Sudo et al.[131] has documented that germ-free mice showed higher plasma levels of adrenocorticotropic hormone and corticosterone following restraint stress, with reduced expression of brain-derived neurotrophic factor in the cortex and hippocampus compared with levels in specific-pathogen-free mice. In addition, the response to stress increased following oral inoculation with enteropathogenic *E. coli*. The enhanced stress response was partially ameliorated with feces from specific-pathogen-free mice at an early stage, but not at a later stage, reinforcing that the establishment of normal commensal bacteria early in life is critical to support maturation of the hypothalamic–pituitary–adrenal axis.

Effects of leaky gut and intestinal and systemic inflammation on the brain

Convincing data are accumulating indicating that the brain (including the regions separated from the

immune system by the blood–brain barrier) is significantly affected by sustained peripheral inflammation, which may activate microglia and perivascular macrophages, leading to a neuroinflammatory status.[132]

Of note, neonatal microglia are more active and more easily primed than adult microglia, owing to higher expression of major histocompatibility complex (MHC) II, CD86, and CD40, whereas perivascular macrophages (expressing mannose receptor CD163) with high MHC II expression are adapted to present antigens to peripheral T cells in the neonatal period.[132–134] In addition, $CD4^+$ T cells can traffic into the brain and may differentiate into T_H1, T_H17, and T_H2 types, depending on microenvironment. T cells are recognized as having a critical role in the crosstalk between the innate and the acquired immune systems, influencing brain inflammation.[135] It is also known that microglia can express mRNA for all Toll-like receptors except Toll-like receptor 10 and therefore can recognize pattern-recognition receptors, which can be especially important in the immature neonatal brain.[136]

In addition, inflammatory responses in the neonatal period may cause increased blood–brain barrier permeability,[137] with white matter injury occurring even during subclinical inflammation,[138] which could facilitate peripheral T-cell and monocyte/macrophage traffic to the brain. Neonatal systemic inflammation may have a lasting effect on behavior[60,139] and cause neuropsychiatric disorders in adulthood.[132]

Role of LPS. Following luminal bacterial translocation, LPS, found in the outer lipid bilayer in the cell wall of gram-negative bacteria, is a strong mediator of intestinal inflammation and activates the mucosal immune system. Lipopolysaccharide is released during bacterial proliferation and as a byproduct of bacterial lysis. Once LPS is released, the lipid A portion of the molecule is exposed and elicits biological responses.[140] If LPS is not neutralized by the gut immune response, it may enter the bloodstream and circulate systemically. It is still unclear how peripheral LPS (or LPS-induced proinflammatory cytokines) leads to neuroinflammation. Cytokines may act directly on neuronal receptors, either following active transport across the blood–brain barrier[141] or after passive diffusion in the circumventricular organs (not associated with the blood–brain barrier). Since LPS cannot easily enter the blood–brain barrier, systemic proinflammatory cytokines (like tumor necrosis factor-α) may mediate the inflammatory signaling effect to the brain, since these cytokines can cross the blood–brain barrier from the peripheral circulation.[142] However blood–brain barrier endothelial cells may respond to LPS and cytokines by producing interleukin 6 and releasing it to the brain tissue.[143] Furthermore, leptomeningeal cells may have a role in inducing proinflammatory responses in the brain after systemic inflammation.[144]

Recently, it was demonstrated that acute injection of a septic dose of LPS intraperitoneally could lead to breakdown of the blood–brain barrier and increased brain levels of proinflammatory cytokines with activated microglia in an experimental model.[145] Systemic LPS (indirectly, by inducing blood–brain barrier epithelial cells or proinflammatory cytokine release) could activate microglia and cause neuroinflammation in the adult[142] and aged brain.[146,147] This may predispose to neurodegeneration,[142] whereas aging reduces the responsiveness to the interleukin 4–induced M2 microglia phenotype.[147] In addition, peripheral inflammation (induced by carrageenan injection into the paw of rats) can exacerbate the loss of dopaminergic neurons and the increase in brain inflammatory markers caused by intranigral injection of LPS.[148] It has been suggested that disruption of the intestinal mucosa by increased translocation of gram-negative bacteria (including *Hafnei alvei*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Pseudomonas putida*, *Citrobacter koseri*, and *Klebsiella pneumoniae*) is associated with major depression.[149] However, it is still uncertain whether low doses of LPS can cause changes in the blood–brain barrier significant enough to cause microglial activation and neuroinflammation over extended periods of time.

As documented recently, low levels of systemic LPS do not seem to cause impairments to the blood–brain barrier or induce microglial activation and neuroinflammation in neonatal mice.[150] In addition, microglial activation is not always associated with exacerbated brain injury. It may be neuroprotective and contribute to the regenerative remodeling following low levels of circulating LPS.[151] Nevertheless, it is unclear whether enteric infections and undernutrition affect these protective responses. It is noteworthy that, in critical periods of brain development, microglia may be primed by a systemic inflammatory response early in life (or prenatally) and later become overactive during neurodegenerative processes that occur with aging, increasing the risk of neuronal death.[152] Another intriguing possibility is that enteric pathogens could influence brain neuroinflammation by releasing factors carried by retrograde axonal transport via the enteric nerve plexus.[153]

Restoring balance in the gut–liver–brain axis (micronutrients and the intestinal microbiota)

The influence of early-life malnutrition and enteric infection on the intestinal microbiome (perhaps especially in the small intestine) is being increasingly recognized as an important factor modulating intestinal adaptive

responses such as unbalanced innate (over-reactive) immune responses and impaired intestinal barrier function. If these deleterious effects occur during a time of profound changes in brain plasticity, such as actively ongoing myelination, neurogenesis, and synaptogenesis processes, they may negatively affect memory storage, executive function, and language circuitry maturation, ultimately leading to poor cognition.[154,155] A compelling therapeutic strategy is to use nutritional supplementation that provides both preventive and curative benefits to children residing in areas where malnutrition and enteric diseases are endemic. One important rationale is to use key gut-trophic nutrients (e.g., zinc, glutamine, citrulline, arginine) to improve the intestinal barrier function, thereby reducing exposure of the intestinal lamina propria to pathogenic bacterial translocation. Apart from the nutritional effect per se, such nutrients/micronutrients may have an advantage in terms of interacting positively with the intestinal microbiota.

L-arginine and citrulline. L-arginine has been shown to ameliorate intestinal epithelial barrier function by improving tight junctions[156] and to diminish luminal intestinal bacterial translocation following intestinal barrier impairment.[157–159] Recently, it has been shown that supplemental dietary L-arginine interacts with the intestinal microbiome to activate the immune system by enhancing intestinal Toll-like receptor signaling and the expression of secretory immunoglobulin A, mucins, and defensins.[160] Arginine given as oral supplementation is converted to ornithine and urea by the liver, the former being a precursor of polyamines (a known cell proliferative factor[161]) by the activity of the ornithine decarboxylase enzyme. In addition, intestinal bacteria may convert dietary arginine to polyamines, which may aid in recovery of the intestinal mucosa following injury.[162,163] Intestinal polyamines were found to be a procognition factor in elderly mice.[162] However, one pitfall of oral arginine supplementation is that arginine may induce increased circulating levels of arginase and therefore lead to arginine breakdown and poor bioavailability in the target tissue.[164,165] One way to overcome this problem is by oral citrulline supplementation. Citrulline, a precursor of arginine, is not known to induce excessive levels of arginase and is not subject to liver metabolism. In addition, citrulline supplementation was found to be beneficial in models of chemotherapy-induced intestinal mucositis and intestinal obstruction in mice.[166,167]

Glutamine, probiotics, and zinc. Glutamine has also been found to interact with the intestinal microbiome, improving host innate immune responses[168] in addition to having well-known effects in protecting the intestinal barrier function and improving intestinal bacterial translocation in models of intestinal injury.[169] Interestingly, dietary glutamine supplementation favored growth of Bacteroidetes instead of Firmicutes, improved Toll-like receptor 4 responses, and increased the level of antibacterial substances in the small intestine.[168] Increased Firmicutes populations in the gut have been associated with obesity.[170] In addition, probiotics such as *Bifidobacterium*, *Saccharomyces*, and *Lactobacillus* have been shown to protect the intestinal barrier function and stimulate host immunity early in life, which may have potentially long-term effects during the life span.[171] Lactobacilli in the gut were found to be protective against *Shigella* diarrhea.[101] Probiotics play a key role in regulating the intestinal microbiota and may have procognition effects.[172] Although findings have been more contradictory, zinc supplementation has also been found to benefit the intestinal microbiota[173,174] and to modulate the intestinal inflammatory responses,[175,176] even reducing the virulence of pathogenic *E. coli*.[177,178] Zinc supplementation is still the only nutritional therapy clearly recognized to protect children with diarrheal diseases in low-income countries.[179] These findings highlight the benefits of certain nutrients in restoring or maintaining intestinal barrier function, but how these nutrients interact with the early-life intestinal microbiota when enteric pathogens are present is largely unknown. More studies are warranted to dissect how these interactions influence cognitive development in children and infants in settings of enteric infection and malnutrition.

CONCLUSION

It is increasingly recognized that a prolonged state of environmental enteropathy, an intestinal inflammatory condition that may profoundly limit an individual's full potential and may go chronically unrecognized in children, in a setting of endemic enteric infections and malnutrition is a strong driving force toward poor development. As a malabsorptive condition, environmental enteropathy may also impair the transport of adequate key nutrients to the brain within an important time period of postnatal brain plasticity, especially in the first 2 years of life. This developmental window of time is the very same time when enteric infections and malnutrition prevail in children exposed to fecally contaminated environments, amplifying the vicious cycle. Although more studies have been published recently, there is still an important knowledge gap about how enteric pathogens interact with the early-life intestinal microbiota. These potential effects of environmental enteropathy on early cognitive development in children are still mostly unknown. Long-term cohort studies are key to dissect the determinants of these lasting effects.

Any effective intervention to ameliorate environmental enteropathy should consider the interactions between the intestinal microbiota and the enteric pathogens that may contribute to poor cognition in children.

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