

The Microbiota and Gut-Brain Axis

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ABSTRACT:

The microbiota and gut-brain axis

The ability of gut microbiota to communicate with the brain and hence modulate behavior is an emerging novel concept in health and disease. The enteric microbiota interacts with the host to form essential relationships that govern homeostasis. Although enteric bacterial fingerprint of each individual is quite unique, there appears to be a certain balance that confers individual's health benefits. A developing number of studies demonstrated that the microbiome of the human digestive tract might have had an effect on the elements of the focal anxious framework (CNS), through recognized pathways called the gut-brain axis. Recent data showed that the human microbiome ecosystem interfered with the brain's development, central signaling systems, and behavior. It has been proposed that the disruption of the human microbiome may contribute to the etiology and course of some psychiatric disorders. Therefore, a decrease in the desirable gastrointestinal bacteria would lead to deterioration in gastrointestinal, neuroendocrine, immune functioning and consequently an illness. This review article presents an overview about the main pathways of the gut-brain axis and consequences of stress to the individual components.

Keywords: brain-gut axis, microbiota, probiotics, anxiety disorders

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INTRODUCTION

With a growing appreciation of the healthcare implications of an aging global population obtaining a better understanding of how the bidirectional interaction between the microbiome and gut-brain axis that influences age-related changes in the brain functioning, must be a priority. Recent studies have made it clear that microorganisms (gut microbiome) play an essential role in this communication process, and researchers are now finding that the microbiome-gut-brain axis does not only have an impact on our appetite and metabolism, but also influences our

behaviour, thoughts, and mood (1). The brain does not act in isolation of our body but in response to the needs of our organs. This emerging knowledge about the connections between the brain and the intestinal tract, leads to several new treatment opportunities against brain disorders (e.g., schizophrenia, depression, anxiety, and autism) and metabolic disorders (e.g., irritable bowel syndrome, IBS). But for treating these diseases we need to understand the entire processes of the microbiome-gut-brain axis. This review article shows an overview about the main pathways of the gut brain axis. Furthermore it illustrates consequences of stress to the individual components (2).

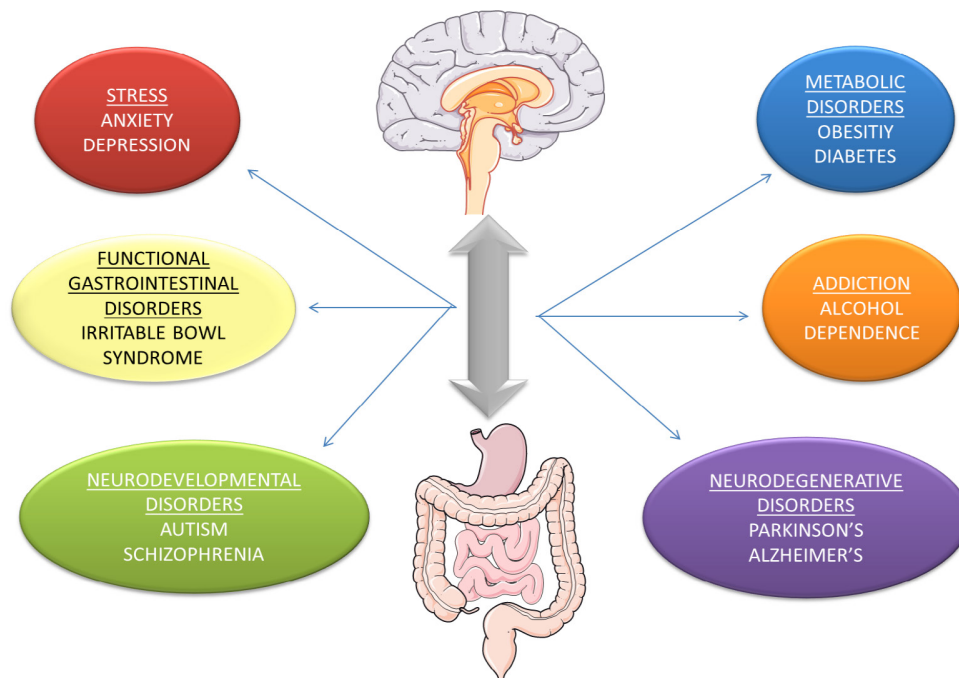


Figure 1: Disorders of the microbiota-gut-brain axis. The axis plays an important role in homeostasis and has been linked to several disorders.

Gut-brain axis and its pathways

The gut-brain axis describes the biochemical signaling between the central nervous system (CNS) and the gastrointestinal tract. The communication of the gut-brain axis occurs directly and indirectly through neuronal, endocrine, or immunological pathways in bidirectional ways (2). The neuronal pathway involves beside the central nervous system also the autonomic nervous system (ANS) and the enteric nervous system (ENS). The ENS is also called 'second brain', because of its size and similarity in complexity, neurotransmitters, and signaling molecules (2). Efferent effects modulate physiological response and immune activity, while afferent neurons convey information to the brain and are responsible for gut reflexes. Interestingly the ENS is also able to work independent from the brain (3). The autonomic nervous system embraces the sympathetic and parasympathetic nervous system. The vagus nerve as part of the parasympathetic nervous system contains about 80% of afferent fibers that lead from the gut to the brain and turns out to play an important role in probiotic treatment (4). For instance *Lactobacillus rhamnosus* is activating the vagus nerve and influences the expression of GABA mRNA

in brain. Bravo et al. found that feeding mice with *Lactobacillus rhamnosus* causes behavior changes only in mice with intact vagus nerve but not in vagotomized ones. Independent of vagal communication is the humoral pathway of the gut-brain axis, which occurs through cytokines, hormones, and neuropeptides (5,6). The hypothalamic-pituitary-adrenal (HPA) axis as one part of the humoral signaling way regulates by its release of cortisol gut movement and integrity likewise vagus as gut permeability and microbiome composition (6). Furthermore the enteroendocrine system follows humoral pathways by delivering the peptide hormone ghrelin. It is mostly released in the mucosal cells of the intestinal tract and has several functions as regulating food intake, and inducing serum concentration of ACTH and cortisol (7). As a third part of the humoral communication, the mucosal immune system acts through endocrine release. It is regulated by the intestinal microbiota and can, if necessary, lead to an immune response via cytokines and immunoglobulins (Ig) as written below (3). Interestingly, three quarter of all human body immune cells are located in the gastrointestinal tract, which illustrates once again the influential function of the human gut (8).

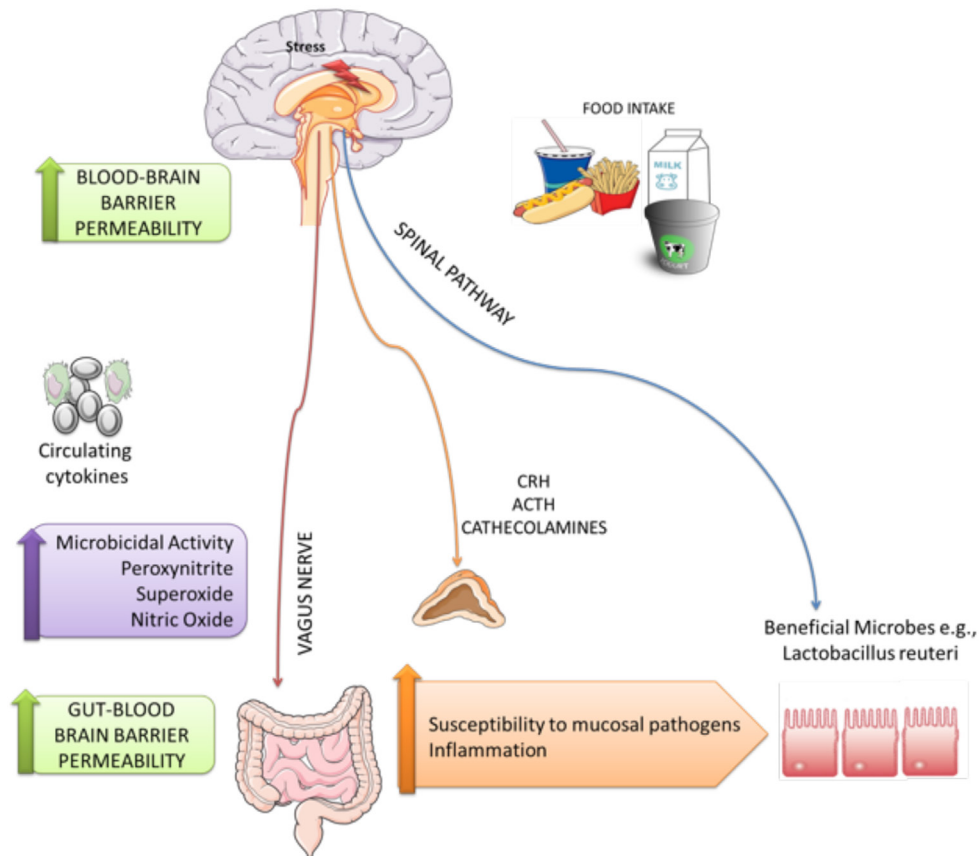


Figure 2: Pathways involved in gut microbiota and the brain. There are many potential pathways such as endocrine (HPA axis), immune (cytokines), and neural (vagus and enteric nervous system) pathways. The gut microbiota, probiotic agents, circulating cytokines and short-chain fattyacids in food have an effect on brain functions. Abbreviations: CRH, corticotropin releasing hormone; ACTH, adrenocorticotrophic hormone.

The innate immune system comes with the toll-like receptors (TLRs), which function as pattern recognition receptor (PRR)(9). TLRs are the gateway to immune response as they recognize molecular patterns, which are linked to pathogens, and initiate a cascade of actions leading to cytokines release and HPA axis activation. Interestingly, Toll-like receptor 4 knockout mice did not respond to gram-negative bacteria with an activation of the HPA (10). Also epithelial and endothelia cells express TLRs. Therefore, TLRs are interesting dueto leaky gut syndrome in which increased permeability of intestinal epithelium cells is impairing the barrier function of the epithelium in the gut (9). Additionally, the tryptophan metabolism has influence on the gut-brain axis. Serotonin (5-HT), as a metabolite of tryptophan, is acting as a biogenic amine neurotransmitter in the body (11) and known to play an important role in mood disorders such as major depressive disorder, anxiety disorder, and schizophrenia (12). Interestingly 95% of 5-HT

releasing cells are located in the gastrointestinal tract (13) and responsible for secretion, sensing, and signaling (9). It shows the importance of the relationship between the gut and the CNS in order to embrace brain disorders based on unbalanced 5-HT levels. It is also activating intrinsic and extrinsic primary afferent neurons (13).

Gut microbiota

The microbiota includes primary bacteria but also viruses and protozoa and forms the so-called commensal microbiome. The lower intestine contains 10¹⁴–10¹⁵ bacteria, that is 10–100 times more bacteria in the gut than eukaryotic cells in the human body (1013) (14). It points out that humans comprise more prokaryotic bacterial cells than actually eukaryotic human cells and what an important role these bacteria play in health and disease. The intestinal microbiota is rich in diversity and composition (15) and

shows an interlinked symbiotic relationship with the host (9). The majority of bacteria in the gut take Firmicutes (such as Lactobacillus, Clostridium, and Enterococcus) and Bacteroides. Actinobacteria (Bifidobacteria), Proteobacteria (Escherichiacoli), Fusobacteria, Verrucomicrobia, and Cyanobacteria are also present but rarely (9). Although the intestinal microbiota is considered relatively stable, some studies have shown a marked variation in the complexity and stability of Bifidobacteria and Lactobacillus populations over a 12-month period and significant alterations in microbiota composition due to environmental factors (6). The colonization of the gut starts with birth therefore the delivery of birth has influence on the microbiota composition. During the first years of life the composition of the microbiota is changing and growing until it gets adult like by the age of three (15). It is shaped by multiple factors including maternal vertical transmission, genetic make up of the individual, diet, medications such as antibiotics, gastrointestinal infections, and stress. Therefore the microbiota of each individual shows a lot variation although there is a stable pattern in every microbiome in healthy population. Furthermore the microbiome of family members is shown to be more similarity when compared with unrelated people (6).

Differences exist between the microbiota composition between the gut lumen and the microbiota composition which lays in close proximity to the mucus layer. For instance, gram negative bacteria as Proteobacteria and Akkermansia muciniphila (Verrucomicrobia), which use mucus as a carbon and nitrogen source, adhere and reside within the mucus layer. This gradient can be altered by factors such as stress (9). Also antibiotic use can change microbiota composition and even leads to behavioral changes. This was found by Bercik et al. when treating mice with antibiotics. While in germ-free (GF) mice no behavior changes are notable, there must be a connection between the microbiome and behavioral alterations (6). This illustrates how the gut microbiota influences CNS at healthy and disease for instance in several of neuroimmune and neuropsychiatric disorders (16).

The CNS in contrast controls the gut microbiome composition through signaling peptides, endocrine and neural pathways. Immune pathways can be turned on in response to altered gut functions. Endocrine and neural pathways can also regulate the secretion of gut epithelial cells. Their secretory products affect the environment of microbiota. This huge influence in behavior can be

explained among other things by the alteration of blood-brain barrier (BBB) permeability and intestinal barrier function (9). An example is the production of short chain fatty acids (SCFA), which can cross the BBB (17). Thus, SCFA are able to interact with the immune system as they can activate the sympathetic nervous system (9).

Also the development of HPA axis is dependent on the microbiome (9). In contrast, the HPA axis can also effect the gut microbiome composition, which illustrates the bidirectional function of the gut-brain axis (6). Microbiota functions as modulation of blood flow, motility, secretion permeability (18). It fortifies the intestinal barrier and induces secretory IgA to limit bacterial penetration into tissues and facilitates nutrient absorption by metabolizing indigestible dietary compounds (16). The gut microbiome is not only defending against pathogen colonization by producing antimicrobial substances, but also the guiding functionality of the immune system.

Explanation BOX

GF mice: *These mice are raised up under special germ free (GF) conditions in isolators. They are without any living microorganism on or in it and therefore with an undeveloped immune system. GF mice are used for research studies about the influence of gut microbiome and probiotics to our body.*

Microbiota: *It describes the collective microbial population, which are resident in the body. Microbiome: Microbiome is the entire genetic material of all the microorganisms in a microbiota of a particular site, for example the human GI tract or the skin.*

Probiotics: *Probiotics are live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host. They maintain intestinal barrier function and support occluding expression, a protein, which builds tight junctions. Probiotics are emerging as potential therapeutics for stress-related disorders.*

Prebiotics: *Substances, which induce growth of probiotics such as Lactobacillus sp. or Bifidobacterium infantis.*

Intestinal barrier

The intestinal barrier is one of the most important components to prevent the body from several diseases. It

has mainly two functions; first, its selective permeability opens the opportunity of a filter function for nutrient absorption. Second, its barrier function prevents toxic and potentially harmful substances, such as antigens, from leaving the intestine and entry to the circulation (19). The intestinal barrier is therefore in permanent exchange with the environment to regulate the up keeping of homeostasis. It is composed of different layers (9).

Villi and microvilli cover the surface of the luminal side of the epithelium cells and can therefore offer a gigantic contact area for the nutrient absorption and water and electrolyte transport. It offers a great entry for toxic substances and microorganisms antigens as well (8). The epithelial cells are connected by tight junctions, which are complex protein structures made of transmembrane proteins, such as claudin, occludin, and tricellulin (9). They keep up a barrier to paracellular diffusion of fluid and solutes. Tight junctions are already detected from week ten, while their functional development continues in postnatal period (9). The epithelium layer exhibit mucosal cells that coat the epithelium by a layer of mucus. On top of the mucus exists and surface active phospholipid layer which is responsible for the hydrophobic and also the size of the mucosal surface (19). Various immunological components as antigens and also non-immunological cells in the mucosal layer, such as secretory IgA and antimicrobial peptides (9), decide if an immune response is necessary to keep up the homeostasis (8) and help to protect against bacterial invasion (9). An unstirred water layer covers the mucosal layer. However, it is unknown which role it plays in permeability but its size seems to be affected by luminal starring (19). The microbiome is able to influence the permeability of the intestinal barrier, for instance through producing SCFA by bacterial fermentation in the gut (20). The SCFA are rapidly absorbed by the colon and especially butyrate can change the expression of tight junction proteins as occludin and have influence over the barrier function of the intestinal epithelial cells (9).

Blood-brain barrier (BBB)

BBB shows some parallels to the intestinal barrier, as it also acts like filter and barrier to protect the CNS from the entry of damaging substances out of the circulation (17). The blood-brain barrier allows passage of water, some

gases and lipid-soluble molecules by passive diffusion and holds selective transporter for glucose as well. Furthermore the BBB prevents the entry of neurotoxins and harmful molecules to the brain and P-glycoprotein helps to carry these substances back to circulation after crossing the BBB. There are two ways of entry; first the paracellular way through tight junctions, which connect the endothelial cells of the blood-brain barrier, and second the transcellular way through the cells of the blood-brain barrier.

The BBB is formed by endothelial cells and glial cells as astrocytes and pericytes adherent to the abluminal side of the microvessels. The endothelial cells differ from other endothelial cells in the body in specific receptors and transporters. Tight junctions function as small barriers of diffusion of intercellular pores and connect the endothelial cells of the blood-brain barrier just as in the intestinal barrier (9,21). They consist of dimer of transmembrane proteins like occludin, claudin, and junctional adhesion molecule (JAM). Their barrier function is given by extremely high electrical resistivity, which averts paracellular barrier entering.

Changes in BBB permeability can be caused by altering tight junction integrity (paracellular way) or by affecting properties or the expression of hormones or other molecular transporters (transcellular way). Tight junction scan be influenced by hormones as glucocorticoids and therefore it can come to alterations in the BBB permeability. Reasons for this are changes in tight junction protein expression caused by glucocorticoids. There are several more peptide hormone that are interacting with the BBB, for example ghrelin and leptin, which are among others responsible for hunger and homeostasis. The permeability of the BBB can be also influenced by cytokines, which are not crossing the BBB from the circulation, but are also expressed in the endothelial cells of the BBB and control the homeostasis of energy transfer (21). Thus the microbiome is able to change permeability. It was shown that the BBB of GF mice are a lot more permeable. Interestingly, it could be shown, that the BBB permeability could restore by fecal transfer of pathogen free mice (17) and the tight junction expression was increased (9). Also SCFA (such as butyrate) are able to normalize the BBB permeability by increasing the expression of tight junction proteins. The SCFA can cross the BBB by using monocarboxylate transporter, which are especially expressed at the BBB (9).

Influence of stress and relationship with depression

Since the gut microbiota is interacting bidirectionally with the gastrointestinal tract, CNS, ANS, and the immune system, alterations of the gut microbiota can influence these components of the gut brain axis. Stress influences the energy homeostasis, intestinal barrier function, and microbiota composition. An unbalanced microbiota of symbionts and pathobionts can trigger inflammation (11, 22) could show that stress raises HPA axis function and immune response in male rats and induces long-term changes in visceral function and sensitivity as well as changes in microbiota diversity and composition. Thus, plasma corticosteroid levels of rats exposed to early life stress turn out to be higher compared to the control group (22). These findings may show an influence of early life on stress-related disorders in adulthood. The most dynamic changes of the microbiota take place in childhood and adolescence. This is also the developing time of the brain and explains why disruptions during this period can have long-term effects on our health, in the gut as well in the brain (11). The increased concentrations of proinflammatory cytokines seen in MDD may also result from interactions with gut microbes. Levels of serum antibodies against lipopolysaccharide from gram-negative enterobacteria are higher in patients with MDD than in controls and cause stress associated with increased gut permeability and bacterial translocation in animal models. Animal studies lead the way in showing that specific strains of bifidobacteria, lactobacilli, or bacteroides may have effects on the brain and behavior (23-25). Also neonatal stress can change composition and diversity of gut microbiota (22). Exaggerated cortisol and adrenocorticotropic hormone (ACTH) response to restraint stress in connection to hyperactivated HPA axis were also found in GF mice and show the link between HPA axis and gut microbiota. Interestingly, anxiety-like behavior in GF mice was decreased compared to specific pathogen free (SPF) mice (26). This finding shows that anxiety-like behavior is not related to cortisol levels (27). Treatment with probiotics during early stress period normalizes basal cortisol levels as *Lactobacillus farciminis* reduces intestinal permeability and prevents HPA hyperactivity (28). In another example *Lactobacillus rhamnosus* reduced depressive-like behavior and can also reverse inflammatory related behavior changes (5).

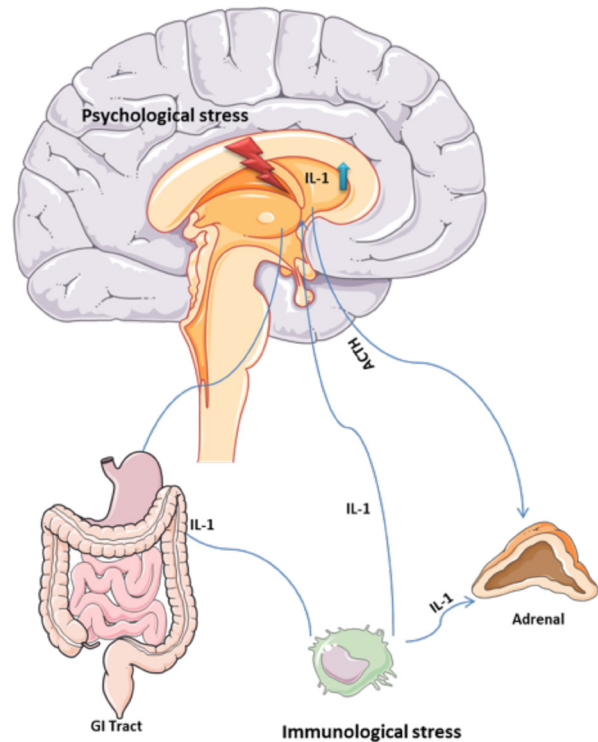


Figure 2: Mechanisms for microbiota and immune signalling, bacterial-derived cell wall components (peptidoglycans). There may be communication routes between gut microbiota and brain, including hypothalamic-pituitary-adrenal axis for endocrine signals (such as ACTH and cortisol), gut immune system (circulation immune signalling cells and cytokines especially IL-1), some neurotransmitters neural regulators synthesized by gut bacteria, and barrier paths including intestinal mucosal barrier and blood-brainbarrier. Abbreviations: GI, Gastrointestinal; ACTH, adrenocorticotropic hormone; IL-1: interleukin 1.

Bifidobacterium infantis seems to be effective in human studies with IBS patients by changing plasma proinflammatory to anti-inflammatory cytokine ratio (29).

Stress is not only influencing the microbiome and the brain, but it also defects the intestinal barrier and lead to increased permeability and mast cell activation (16), which in turn modulates inflammation and immunity (6). Translocation of bacteria across the intestinal mucosa opens a pathway opportunity how stress can influence the CNS (8). This 'leaky gut' describes a loss of barrier function and leads not only to the GI dysfunction but is also found in patients with psychiatric disorders, such as depression and chronic fatigue syndrome. However, it is unclear whether these disorders are cause or consequence of the

increased intestinal permeability (11). Increased permeability helps pathobionts to pass to the systemic circulation through the mucosal layer of the intestinal tract. Once in the circulation, the pathogenic bacteria produce proinflammatory cytokines by which they are activating the immune system in an indirect way and it comes to systemic inflammation and abnormal gut function (11). Furthermore, systemic inflammation can increase intestinal barrier permeability and thus allow translocation of commensal bacteria with further implications for systemic inflammation (9). Stress can also activate neuronal cells of the ENS by translocation of microbiota across the intestinal mucosa (28). Also the BBB permeability is changed by factors as stress or emotions since the tight junction protein expression are able to be increased by glucocorticoides. Up regulated expression of these proteins can strengthen the barrier function of the BBB (21).

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CONCLUSIONS

Recent studies demonstrated that germ-free mice display alterations in stress-responsivity, central neurochemistry and behavior indicative of a reduction in anxiety in comparison to conventional mice. Such research findings offer the exciting proposition that specific modulation of the enteric microbiota might be a useful strategy for stress-related disorders and for modulating the comorbid gastrointestinal disorders such as irritable bowel syndrome and inflammatory bowel disease. All of the current data indicate that there is an increasing need to a better understanding the molecular, cellular and physiological basis of enteric microbiome and gut-brain communication. Future studies will provide insight into the development of novel treatment strategies (including probiotics), for psychiatric disorders and gastrointestinal disorders that are associated with an altered signaling from the bowel to the brain.

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