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Evaluation of the Mechanisms Underlying Amino Acid and Microbiota Interactions in Intestinal Infections Using Germ-free Animals

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Author contributions: HW conceptualized the review; YY, PB, ST and GZ drafted the manuscript; ZW, WC, and WR reviewed and compiled the data and drafted the manuscript; all authors read and edited the manuscript and approved the final manuscript.

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**Abstract:**

Intestinal infectious diseases refer to the inflammatory changes in the intestinal tract caused by pathogens (including bacteria, viruses, fungi, protozoa, or parasites) or their toxic products. A large number of microorganisms colonize the intestinal tract of healthy people, which together with the intestinal epithelium constitute the biological barrier of the intestinal tract to resist infectious diseases. As an “invisible organ,” the intestinal flora is closely related to human nutrition metabolism and intestinal infections. A variety of intestinal flora participates in the nutritional metabolism of amino acids, and the small molecular substances produced by the amino acid metabolism through the intestinal flora can enhance intestinal immunity and resist bacterial infections. In turn, amino acids can also regulate the composition of the intestinal flora, maintain the steady-state of the intestinal flora, protect the intestinal barrier, and inhibit colonization by pathogenic bacteria. As a model animal with a clear microbial background, germ-free (GF) animals can clarify the mechanisms of interactions between intestinal microbes and amino acid metabolism in intestinal infections by combining genetic engineering technology and multi-omics studies. This article reviews related researches on the involvement of intestinal microbes in host amino acid metabolism and resistance to intestinal infections and discusses the advantages of GF animal models for studying the underlying mechanisms. The GF animal model is helpful to further study the intervention effects of amino acid metabolism of targeted intestinal flora on intestinal infections.

**Keywords:** intestinal infection; intestinal flora; germ-free animal; metabolism of amino acids; intestinal barrier; intestinal immunity
Introduction

The clinical symptoms of intestinal infections caused by pathogens (including bacteria, viruses, fungi, protozoa, or parasites) include abdominal pain, diarrhea, mucosal congestion, mucosal erosion, and ulcer formation as evaluated by endoscopy. Pathogenic microorganisms also induce mucosal damage and destruction of the intestinal symbiotic flora, which may cause long-term intestinal dysfunction. When intestinal infections occur, pathogenic bacteria can competitively bind to the intestinal epithelium in conjunction with the intestinal symbiotic flora, thus destroying the intestinal microbial barrier and affecting the integrity of the intestinal mucosal barrier. The intestinal mucosal barrier plays an important role in resisting invasion by pathogenic microorganisms, toxins, and antigens in the gastrointestinal tract. The human body is considered a superorganism composed of host cells and symbiotic flora, in which the number of bacterial genes is approximately 100 times that of the host genes. The intestinal flora is closely related to human health, and a large number of microorganisms colonize the intestinal tract of healthy people, and interact with and depend on other bacteria to keep the balance; these microorganisms can be closely combined with the intestinal epithelium to form the intestinal biological barrier. The intestinal microbial community forms a symbiotic relationship with the body. It participates in the regulation of host health by regulating intestinal functions, metabolism, angiogenesis, and immune functions. Conversely, dysbiosis of the intestinal microbial community is closely related to various diseases, such as cardiovascular diseases, metabolic diseases, mental diseases, and tumors. In addition, intestinal microbes are closely related to the occurrence of infectious diseases. An important function of the intestinal flora is to prevent the colonization of pathogens in the intestine and prevent infectious diseases, which is termed “colonization resistance”. Previous studies have shown that compared with normal mice, the colonization resistance of the intestinal flora of antibiotic-treated mice and germ-free (GF) mice is reduced. In summary, intestinal microbes can prevent infectious diseases by directly inhibiting the colonization by pathogens, maintaining the intestinal barrier, regulating the host immune system, and participating in nutrition metabolism.

Amino acids are an important part of the human diet, which do not only synthesize related proteins, but also promote the production of multiple bioactive molecules that participate in signaling pathways and maintain physiological metabolism. The intestine is considered the main site for the absorption and transport of amino acids in mammals, and it is also responsible for a considerable part of amino acid metabolism by the support of intestinal microbiota. The intestinal microbiota drives amino acids derived from exogenous dietary sources and endogenous tissues for the de novo biosynthesis of proteins. In addition, the intestinal microbiota catabolizes amino acids to produce important functional small molecule metabolites, such as hydrogen sulfide, nitric oxide, polyamines, and indole compounds. As
essential regulators of cellular signal transduction and intermediaries between the host and microbiome, amino acids and their metabolites play a profound role in protecting the intestinal mucosal barrier function, maintaining intestinal microbiota homeostasis, and resisting invasion by pathogens (Figure 1). Furthermore, amino acids are also essential nutrients for the normal growth and proliferation of pathogens, and amino acid deprivation would severely disrupt bacterial physiology and virulence. In addition, a variety of amino acids can be metabolized into short-chain fatty acids (SCFAs) by anaerobic bacteria. For example, glycine, threonine, glutamic acid, and ornithine may be metabolized to acetate, while threonine, lysine, and glutamic acid can be used to synthesize butyric acid, and threonine is involved in the synthesis of propionic acid. SCFAs perform a variety of physiological functions, including regulating epithelial barrier functions, regulating inflammation through the natural immune pathway of the host, and resisting intestinal pathogens. In the last few decades, more attention has been paid to the crosstalk of amino acids between the host and pathogens, and great progress has been made in research on several amino acids, including tryptophan, arginine, and glutamine. Microbial metabolites can coordinate intestinal homeostasis by mediating the interaction between the symbiotic flora and the host immune system. The utilization of amino acids by intestinal bacteria plays a key role in regulating intestinal homeostasis. Therefore, it is important to analyze the function of the microbiome in human beings and clarify the role of intestinal microbes in amino acid metabolism and intestinal infections. GF animals have become an irreplaceable tool for studying the relationship between single bacteria, multi-bacteria, or intestinal flora and the host. Owing to the absence of any detectable live bacteria, viruses, and parasites, GF animals are the most suitable animal models that remove microbial interference. This review summarizes the most recent studies regarding intestinal microbiota-induced amino acid metabolism and the role of common amino acids in the process of intestinal infections. Moreover, we report that GF animal models can be used for research on the interactions between intestinal flora and amino acid metabolism in intestinal infections. It will help to understand the amino acid interactions between host and intestinal microbes and explore their unknown mechanisms of resistance to intestinal infections.

Intestinal microbiota-induced amino acid metabolism and intestinal infections

Arginine

Arginine is a semi-essential amino acid that serves as the precursor for the biosynthesis of proteins and arginine-derived metabolites, such as polyamines, nitric oxide, glutamate, urea, and creatine. Intestinal arginine is involved in three predominant metabolic pathways: (i) direct catabolism into nitric oxide through catalysis by nitric oxide synthases; (ii) synthesis of urea and creatine through catalysis by arginase and arginine: glycine amidinotransferase and
simultaneous production of ornithine; and (iii) synthesis of polyamines by providing intermediate ornithine and agmatine. Intestinal epithelial cells highly express several arginine metabolic enzymes (eg, nitric oxide synthase and arginase), and therefore, 40% of arginine in the intestinal lumen is catabolized in the first-pass of the intestine.\textsuperscript{23,24} Remarkably, the intestine harbors a large number of amino acid-fermenting bacteria, especially in the large intestine. They utilize amino acids for normal growth and feed the processed proteins or metabolites back to the host, thereby improving the bioavailability of amino acids. Arginine transporters and arginine metabolic pathways have been identified in multiple bacteria present in the intestine, such as \textit{Escherichia coli}. \textit{E. coli} has an integrated and complex genome network that encodes essential arginine metabolic enzymes and transporters, such as arginine decarboxylase (\textit{adiA}), ornithine decarboxylases (\textit{speC} and \textit{speF}), and an arginine export pump (\textit{argO}).\textsuperscript{25,26} Differences in intestinal microbiota composition determine its capacity for arginine metabolism. For example, Indian women have lower endogenous arginine flux but higher intestinal arginase activity than Jamaican women in the fasting state. Correlation analysis revealed that it may correlate with the abundance of fecal \textit{Prevotella} (positive correlation) and \textit{Bacteroides} (negative correlation).\textsuperscript{27} In addition, arginine plays an important role in shaping the intestinal microbiota composition among various species. Our previous study found that 0.5% arginine supplementation increased the relative abundance of jejunal \textit{Lactobacillus} and ileal \textit{Streptococcus} but decreased the relative abundance of ileal \textit{Lactobacillus} in mice.\textsuperscript{28} Moreover, 2.46% arginine supplementation increased the relative abundance of colonic \textit{Barnesiella} and \textit{Bacteroides} but decreased \textit{Akkermansia} in mice.\textsuperscript{29} In addition, 0.3% arginine supplementation increased the relative abundance of ileal \textit{Lactobacillus} in broiler chickens.\textsuperscript{30} Although these studies showed different alterations in intestinal microbiota composition because of differences in arginine dosage, experimental animals, and intestinal segments, these results indicate that elevated arginine levels may provide a beneficial environment for the growth of certain microbiota. Indeed, \textit{Lactobacillus} is a potential arginine-favored bacterium in the intestine, as an elevated level of ornithine, which is derived from arginine metabolism through the arginine deiminase pathway, is observed in \textit{Lactobacillus}-rich mice.\textsuperscript{31}

Thus far, it has been fully elucidated that arginine exerts an important protective function against infection by various intestinal pathogens, such as \textit{Clostridium perfringens}, \textit{Salmonella typhimurium}, and \textit{E. coli}.\textsuperscript{30,32,33} Clearly, arginine protects against intestinal infections by improving the intestinal innate immunity, which is characterized by elevated expression of intestinal immune factors (eg, plgR, Mucin 2, CRS1C, and Reg3γ), intestinal tight junction proteins (claudin-1 and occludin), and intestinal secretory IgA (SIgA) levels.\textsuperscript{32,33} In macrophages, arginine availability determines nitric oxide production by restricting the activity of inducible nitric oxide synthase (iNOS).\textsuperscript{34} Nitric oxide is an important antimicrobial
that is easily oxidized into reactive nitrogen intermediates and it inactivates several key metabolic enzymes (e.g., aconitase and ribonucleotide reductase) to kill pathogens. Interestingly, iNOS seems to prefer citrulline-derived arginine over imported arginine sources for nitric oxide synthesis, and the combined supplementation of arginine and citrulline results in a large increase of the total nitric oxide production in macrophages. In addition to targeting the immune system of the host, arginine also exerts an antimicrobial function by maintaining homeostasis of the intestinal microbiota. The composition of intestinal microbiota profoundly affects the outcome of intestinal colonization by pathogenic bacteria, and a dysbiotic intestinal microbiota is more susceptible to enteric pathogenic bacteria, such as *E. coli*, *Listeria monocytogenes*, *Citrobacter rodentium*, and *Clostridium difficile*. Wu et al. reported that dietary arginine supplementation increases the ileal microbiota population and SIgA production in antibiotic cocktail-treated mice, which further improves mucosal immunity of the host. Collectively, arginine maintains intestinal health during invasion by enteric pathogenic bacteria by both improving intestinal innate immunity and maintaining intestinal microbiota homeostasis.

**Glutamine**

Glutamine is a vital conditionally essential amino acid that plays multiple roles in the regulation of nutritional metabolism, immune responses, cellular signaling pathways, and oxidative stress. In mammals, glutamine is also used as the predominant fuel for cell proliferation, such as in the intestinal mucosa, indicating that intestinal glutamine metabolism is crucial for intestinal function. Approximately 30% of the glutamine source is catabolized in the intestine. Intestinal epithelial cells transport luminal free glutamine through SLC1A5, and intracellular glutamine: i) participates in the nucleotide biosynthesis directly; and ii) is converted to glutamate and downstream α-ketoglutarate by glutaminase and aminotransferases or glutamate dehydrogenase, and enters the tricarboxylic acid cycle. Besides enterocytes, the intestinal microbiota also utilizes glutamine as the primary nitrogen source for optimal survival and growth, and specific alterations in microbial composition can profoundly influence the entire glutamine metabolism in the intestine. For example, a recent study found that, compared with control individuals, fibromyalgia patients have higher glutamate and glutamine levels in the serum, which is closely relevant to the decreased abundance of intestinal microbiota, including *Bacteroides*, *Bifidobacterium*, *Eubacterium*, Lachnospiraceae, and Ruminococcaceae. As expected, the genes responsible for glutamine metabolism (e.g., *glnA* and *glsA*) are diminished significantly in these bacteria, which indicates that intestinal microbiota-induced glutamine metabolism is attenuated and thereby causing elevated systemic glutamine levels in fibromyalgia patients. Intriguingly, a previous study found that glutamine flux can also modulate the utilization of other related amino acids, such as lysine, leucine, valine, ornithine, and serine, by the intestinal microbiota. One
potential mechanism is that glutamine alters the intestinal microenvironment and influences the intestinal microbiota composition,\textsuperscript{46} which might further modulate the production of glutamine-related metabolites, such as nitrogenous compounds, to initiate the signaling pathways responsible for other amino acid metabolisms.

Glutamine is a crucial nutrient for maintaining the intestinal function, especially in the regulation of intestinal barrier integrity. The intestinal barrier, which consists of commensal microbiota, mucus, intestinal epithelial cells, and immune cells, is the most important defense for preventing invasion by intestinal pathogens. Our previous study demonstrated that dietary supplementation with glutamine increased both IgA\textsuperscript{+} plasma cells and SIgA levels in the ileum of mice, and the underlying mechanism involves the modulation of glutamine availability to the intestinal microbiota.\textsuperscript{47} Moreover, numerous studies have confirmed that glutamine increases the mRNA and protein expression levels of mucin 2.\textsuperscript{48} Mucin 2, as the main component of intestinal mucus, prevents pathogens from reaching intestinal epithelial cells, which is unsurprising because glutamine is an important N-acetyl-glucosamine and N-acetyl-galactosamine donor for mucin biosynthesis.\textsuperscript{49} In addition to acting on mucus, glutamine plays an important role in maintaining intestinal permeability. For instance, glutamine supplementation rescues the decreased intestinal permeability caused by toxins, stress, and pathogen infection,\textsuperscript{50,51} and glutamine deprivation increases permeability and decreases the expression of tight junction proteins by activating the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in Caco-2 cells.\textsuperscript{52} During intestinal infections, pathogens escape the immune system of the host by driving the intestinal amino acid metabolism.\textsuperscript{53,54} We have found that glutamine concentrations are highly reduced in the ileum of piglets during enterotoxigenic E. coli (ETEC) infections.\textsuperscript{40} Based on the regulatory role of glutamine in innate immunity, we conclude that ETEC competes with the host for the glutamine source to support its normal growth and weaken the innate immunity of the host for successful colonization, which is evidenced by the altered innate immune response during ETEC infections.\textsuperscript{55} In summary, glutamine modulates the intestinal innate immunity, and glutamine availability in the host is one of the determinants that affects pathogen infection outcome.

\textit{Tryptophan}

Tryptophan is an essential amino acid that contains an indole structure, and it serves as the fundamental synthetic precursor of multiple molecules, such as serotonin, melatonin, indole, tryptamine, kynurenine, and nicotinamide adenine dinucleotide. Dietary tryptophan is the sole source of tryptophan in humans and animals. The majority of tryptophan sources are taken up in the small intestine or metabolized through the kynurenine pathway and serotonin production pathway by intestinal cells. The remaining tryptophan enters the large intestine and is further degraded by the intestinal microbiota. The microbiota-induced tryptophan
metabolism is well summarized elsewhere.\textsuperscript{56,57} There is a complex connection of tryptophan metabolism between the host and the intestinal microbiota. The bioavailability of tryptophan in the host determines the outcome of bacterial tryptophan catabolism, and certain genes of the host, such as caspase recruitment domain 9 (\textit{CARD-9}),\textsuperscript{58} affect the production of bacterial tryptophan metabolites by modulating the composition of the intestinal microbiota. Although the intestinal microbiota only catabolizes a few tryptophans from dietary sources, the intestinal microbiota-fermented tryptophan metabolites (e.g., kynurenine, serotonin, indole, tryptamine, indole aldehyde, indole lactic acid, acrylic acid, and indole propionic acid) are crucial signaling molecules for mediating intestinal homeostasis. For example, fecal microbiota transplantation elevates the colonic indole acetic acid levels of piglets by altering the microbiota composition, which further modulates the aryl hydrocarbon receptor (AhR)-IL-22 axis to protect against inflammation induced by lipopolysaccharide, and the genus \textit{Micromonospora} is the potential target microbiota that drives indole acetic acid production.\textsuperscript{59} Alexeev et al. reported that oral indole-3-propionic acid can protect mice from dextran sodium sulfate-induced colitis.\textsuperscript{60} In addition, microbial tryptophan metabolites have emerged as key regulators of the gut-brain function, because these metabolites can cross the blood-brain barrier and directly signal the central nervous system. Previous studies have demonstrated that indole propionic acid, a tryptophan metabolite biosynthesized by \textit{Clostridium sporogenes}, can activate AhR signaling in astrocytes and inhibit inflammation of the central nervous system, but intestinal microbiota dysbiosis or AhR agonist treatment significantly increased the experimental autoimmune encephalomyelitis score of mice.\textsuperscript{61,62}

As the precursor substrate of numerous molecules, especially AhR ligands, tryptophan exerts important functions during intestinal infections. Tryptophan can be catabolized into a variety of AhR ligands by both the intestinal microbiota and intestinal cells, which activates the AhR-dependent signaling of immune cells and affects the intestinal immune response. For instance, the bacterial tryptophan metabolite indole-3-aldehyde increased resistance to \textit{Candida albicans} infection and IL-22 production in colonic NKp46\textsuperscript{+} cells of WT mice, while analogical results failed to be observed in \textit{Ahr}\textsuperscript{-/-} mice.\textsuperscript{63} Indole-3-lactic acid, another bacterial tryptophan metabolite produced by \textit{Lactobacillus reuteri}, is able to promote the differentiation of CD4\textsuperscript{+} T cells to CD4\textsuperscript{+}CD8\textsuperscript{αα} by activating AhR signaling and downregulating transcription factor ThpoK.\textsuperscript{64} Wlodarska et al. reported that \textit{Peptostreptococcus russelli} can improve the intestinal epithelial barrier function and reduce inflammatory signals by metabolizing tryptophan to indole acylic acid (AhR agonist), thus reducing the susceptibility to colitis.\textsuperscript{65} In addition, similar to arginine and glutamine, tryptophan also has a protective role in the intestinal barrier: animal and cellular studies showed that tryptophan can upregulate expression of tight junction proteins\textsuperscript{66,67} and reduce the intestinal permeability,\textsuperscript{68} which collectively contribute to fortifying the intestinal barrier.
Notably, in addition to affecting the immune response, several tryptophan metabolites are potential antimicrobial agents. Indole and its derivatives are regarded as new quorum-sensing molecules that have a negative regulatory role in biofilm formation and virulence gene expression during *Listeria monocytogenes* growth. Thus, intestinal tryptophan metabolism has biologically important roles in maintaining the intestinal function, especially in modulation of the immune system.

Thus, it is necessary to study the relationship between intestinal infections and the interaction of bacterial flora and amino acid metabolism through experimental models and to analyze the resistance of bacterial flora and amino acid metabolism to intestinal infections in terms of correlation and causality. Presently, GF animal models, flora transplantation technology, and genetically engineered animal sterilization technology are universally adopted to study the interactions between amino acid metabolism and intestinal microbes and their impact on intestinal infections.

**GF animal models contribute to clarifying the mechanism of amino acid interactions between host and microorganisms during intestinal infections**

Amino acids can be metabolized by intestinal microbes, releasing metabolites such as ammonia, hydrogen sulfide, polyamines, phenols, and indoles in the intestinal cavity, among which some compounds (such as indole derivatives) can maintain intestinal barrier function. Intestinal bacteria play a key role in regulating the utilization of amino acids and intestinal homeostasis. However, the metabolic mechanisms of these amino acids in GF animals remains largely unknown, and the application of GF animals in exploring the interactions of amino acid metabolism and intestinal microbiota during infections needs to be further improved. Thus, it is necessary to integrate GF animal technology and genetic engineering technology to analyze the function of human beings as superorganisms and study the whole organism completely and deeply from the host (genome) and symbiotic flora (metagenome), both of which are indispensable. With GF animal technology for flora knockout and transplantation, the flora related to amino acid metabolism and intestinal infection resistance can be determined. Similarly, in a GF animal model combined with gene knockout and transgene technology, the relationship between intestinal infections and the interaction between intestinal flora and amino acid metabolism-related genes can be studied. Thus, we put forward the following three points of thinking, as shown in Figure 2.

1) As animals with a clear microbial background, GF animals are the most suitable models for studying the functions of intestinal microbes. Comparing the differences in intestinal microbiota composition between GF and specific-pathogen-free (SPF) animals, it is more precise to analyze the effect of flora on amino acid metabolism and demonstrate its role in
metabolizing amino acids. In addition, amino acid interventions in infected SPF and GF animal models contribute to clarifying the mechanism of amino acid-induced infection resistance.

2) Taking GF animals as recipients, transplantation of bacterial flora, a single strain or multiple strains together can be performed to study the changes in host amino acid metabolism after bacterial flora reconstruction, thereby clarifying the relationship between amino acid metabolism and bacterial flora or strains and directly confirm the mechanism underlying the effect of intestinal flora on host amino acid metabolism and its pathway. In addition, an intestinal infection model can be established using GF animals, in which intestinal flora is inoculated and subsequently amino acid interventions are performed to clarify the interaction between intestinal flora and amino acid metabolism and the mechanism of resistance to intestinal infections. After effective treatment against intestinal infections, a combination of metagenomics, metabolomics, and culturology can be used for the function-oriented screening of microorganisms related to intestinal infection resistance.

3) GF animal and genetically engineered animal technology can be organically combined to clarify the mechanisms by which flora exerts metabolic functions by interacting with host amino acid metabolic functional genes and clarify whether the interaction between flora and functional genes interferes with amino acid metabolism. Based on genetically engineered GF animals, a functional flora transplanted amino acid intervention intestinal infection model can be constructed and combined with genomics, microbiology, and metabolomics methods to study the interaction between intestinal microbes and genes mediating amino acid metabolism in intestinal infectious diseases. To promote studies on the effect of amino acid metabolism and intestinal microbiome on resistance against intestinal infections, it is necessary to fully strengthen the integration of modern technologies and the integration of different disciplines, such as disease model systems based on GF animals and intestinal microbial culture omics, as well as the combination of genetically engineered animal sterilization and flora transplantation technology. Taken together, the core point of this review is to thoroughly compare differences in amino acid metabolism between GF and SPF animals and evaluate the transplantation of flora or specific strains to GF animals, the sterilization of genetically engineered animals, and the construction of intestinal infection models and finally use a combination of multi-omics methods to research the interaction between function-oriented microbes and amino acid metabolism in intestinal infectious diseases.

Summary

In this review, the amino acid interaction between the host and the intestinal microbes are described in detail. Specific amino acids and their metabolites are used as regulators to
regulate immune cell differentiation, promote the secretion of immunoglobulins and cytokines, enhance the intestinal barrier function by increasing the expression of tight junction proteins and reducing intestinal permeability, thereby maintaining the balance in intestinal microflora, and resisting intestinal infections. Amino acid intervention causes changes in the structure and function of intestinal flora and affects intestinal infections. Similarly, the metabolites of amino acids produced by the intestinal flora are also closely related to intestinal infections. As model animals with a clear microbial background, GF animals are not only used to study the interaction of amino acids between the host and intestinal microbes, and analyze the mechanism of amino acid metabolism changes under sterile conditions, but also to construct various models, such as gnotobiotic animals inoculated with flora models, genetically engineered animal sterilization models, and intestinal infectious disease models. These will enable us to study the mechanism by which intestinal functional flora interacts with amino acid metabolism and related functional genes to interfere with intestinal infections. GF animals combined with microbiological methods can promote the study of amino acid metabolism and microbial interaction to resist intestinal infections. In addition, it can be used for the function-oriented screening of microorganisms related to amino acid metabolism and resistance to intestinal infections, which has important implications for basic scientific research and clinical transformation applications. In the future, based on GF animals, microbiology and amino acid nutrition metabolism will be organically integrated, and function-oriented screening of specific microorganisms will be used in nutrition metabolism to interfere with intestinal infections. This will help promote the research and application of nutrition microbiomes against intestinal infections and other critical diseases, strengthen the basic research on the role of nutritional microbiota against diseases, and promote the application of functional microorganisms and amino acid nutrition in clinical transformation.

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**Figure 1. Protective role of amino acids in intestinal infections.** The intestine is the main location of amino acid absorption and transportation, and the amino acid source is transported to the systemic tissues or organs. In addition, amino acids are partially catabolized by the intestinal cells and commensal microbiota. Amino acids and their metabolites are utilized as regulatory factors to (i) regulate the differentiation of immune cells and promote the secretion of immunoglobulins and cytokines, as seen in methionine, serine, glutamine and γ-aminobutyric acid; (ii) fortify intestinal barrier functions by increasing the expression of tight junction proteins and reducing intestinal permeability; examples include glutamine, tryptophan, arginine, glycine, and glutamate; and (iii) maintain the intestinal microbiota homeostasis, such as arginine, γ-aminobutyric acid, tryptophan, serine, and aspartate.

![Diagram of intestinal microbiota and amino acids](image_url)

**Figure 2.** Use of germ-free animals to study the mechanistic details of interaction between the intestinal microbiota and host amino acids and its impact on resistance against intestinal infections.

