8 Impacts of Amino Acids on the Intestinal Defensive System

Wenkai Ren, Peng Bin, Yulong Yin, and Guoyao Wu

Abstract

The intestine interacts with a diverse community of antigens and bacteria. To keep its homeostasis, the gut has evolved with a complex defense system, including intestinal microbiota, epithelial layer and lamina propria. Various factors (e.g., nutrients) affect the intestinal defensive system and progression of intestinal diseases. This review highlights the current understanding about the role of amino acids (AAs) in protecting the intestine from harm. Amino acids (e.g., arginine, glutamine and tryptophan) are essential for the function of intestinal microbiota, epithelial cells, tight junction, goblet cells, Paneth cells and immune cells (e.g., macrophages, B cells and T cells). Through the modulation of the intestinal defensive system, AAs maintain the integrity and function of the intestinal mucosa and inhibit the progression of various intestinal diseases (e.g., intestinal infection and intestinal colitis). Thus, adequate intake of functional AAs is crucial for intestinal and whole-body health in humans and other animals.

Keywords

Amino acids · Intestinal microbiota · Tight junction · Macrophages · T cells

Abbreviations

AAs	amino acids
AJ	adheren junction
Akt	protein kinase B
AMPK	AMP-activated protein kinase
Ang4	RNase angiogenin 4
APRIL	a proliferation-inducing ligand
BCAAs	branched-chain amino acids
CaMKK2	calcium/calmodulin-dependent kinase 2

W. Ren
Guangdong Provincial Key Laboratory of Animal Nutrition Control, Institute of Subtropical Animal Nutrition and Feed, College of Animal Science, South China Agricultural University, Guangzhou, China

P. Bin
Jiangsu Co-Innovation Center for Important Animal Infectious Diseases and Zoonoses, Joint International Research Laboratory of Agriculture and Agri-Product, Safety of Ministry of Education of China, College of Veterinary Medicine, Yangzhou University, Yangzhou, China

Y. Yin
Laboratory of Animal Nutrition and Health and Key Laboratory of Agro-Ecology, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, China

G. Wu (✉)
Department of Animal Science, Texas A&M University, College Station, TX, USA
e-mail: g-wu@tamu.edu

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Crs1c cryptdin-related sequence 1c
DAO D-amino acid oxidase
DSS dextran sulfate sodium
ERK extracellular regulated protein kinases
ETEC enterotoxigenic *Escherichia coli*
FcRn neonatal Fc receptor
GABA gamma-aminobutyric acid
IELs intraepithelial lymphocytes
IFN interferon
IL interleukin
IR ischemia/reperfusion
MAPK mitogen-activated protein kinase
mTORC1 mechanistic target of rapamycin complex 1
NK natural killer
PI3K phosphatidylinositol 3′ -kinase
ROS reactive oxygen species
S6K1 ribosomal protein S6 kinase 1
Sirt1 sirtuin-1
TEER transepithelial electrical resistance
TJ tight junction
TNF tumor necrosis factor
ZO zonula occludens

8.1 Introduction

Interactions with pathogens and toxins are a fact of life for almost all animals, and this is more pronounced in the intestine than any other organs. The small intestine is responsible for nutrient digestion and absorption (Wu 2018). In addition, the gut is the home to a diverse community of indigenous microorganisms. Thus, both the small intestine and the large intestine are constantly exposed to various antigens from food and water, as well as a large number of bacteria that coexist in the intestinal lumen. The gastrointestinal tract has evolved with a sophisticated barrier defense system to protect against this exposure and to distinguish "self" from "foreign". This defense system includes indigenous commensal microorganisms, epithelial layer, and the lamina propria (Fig. 8.1). Intestinal microbiota is associated with the intestinal defensive system through its regulation on intestinal or systemic innate and adaptive immunities (Honda and Littman 2016; Thaiss et al. 2016), as well as direct effects on pathogens via colonization resistance or competition for nutrients (Endt et al. 2010; Seekatz and Young 2014). The epithelial layer includes absorptive enterocytes, hormone-secreting enteroendocrine cells, mucus-secreting goblet cells, antimicrobial-secreting Paneth cells, intraepithelial lymphocytes (IELs), microfold cells, and dendritic cells. The lamina propria harbors various immune cells, such as dendritic cells, neutrophils, macrophages, B lymphocytes (B cells), T lymphocytes (T cells), and fibroblasts. Based on published studies (Johansson and Hansson 2016; Mukherjee and Hooper 2015; Pabst et al. 2016), the intestinal epithelium produces and releases secretory IgA, antimicrobial proteins and mucins in a cell-specific manner.

Recent years have witnessed growing interest in the biochemistry and physiology of amino acids (AAs) in mammals, such as arginine, glutamine, glycine, and tryptophan (Fan et al. 2019; Le Floc'h et al. 2018; Hou and Wu 2017; Wu 2013). Notably, dietary contents of AAs are crucial for intestinal physiology, especially the intestinal defensive immune (Li et al. 2007; Ren et al. 2016a, b). The review highlights our current understanding of the influences of dietary AAs on intestinal defensive system in humans and animal models, including intestinal microbiota, cells in the epithelial layer and immune cells in the lamina propria.

8.2 Amino Acids and Intestinal Microbiota

Intestinal microbiota is present in virtually any metazoans, ranging from invertebrates to vertebrates. It affects numerous physiological functions of the gut (Lee and Hase 2014; Ren et al. 2016b; Subramanian et al. 2014; Thaiss et al. 2016) and is linked to the pathogenesis of various diseases (Anhe et al. 2014; Lee and Hase 2014; Louis et al. 2014; Qin et al. 2014; Thaiss et al. 2016) through the microbiome and its metabolic products (Lee and Hase 2014; Ren et al. 2016d). Intestinal microbiota has critical roles in intesti-

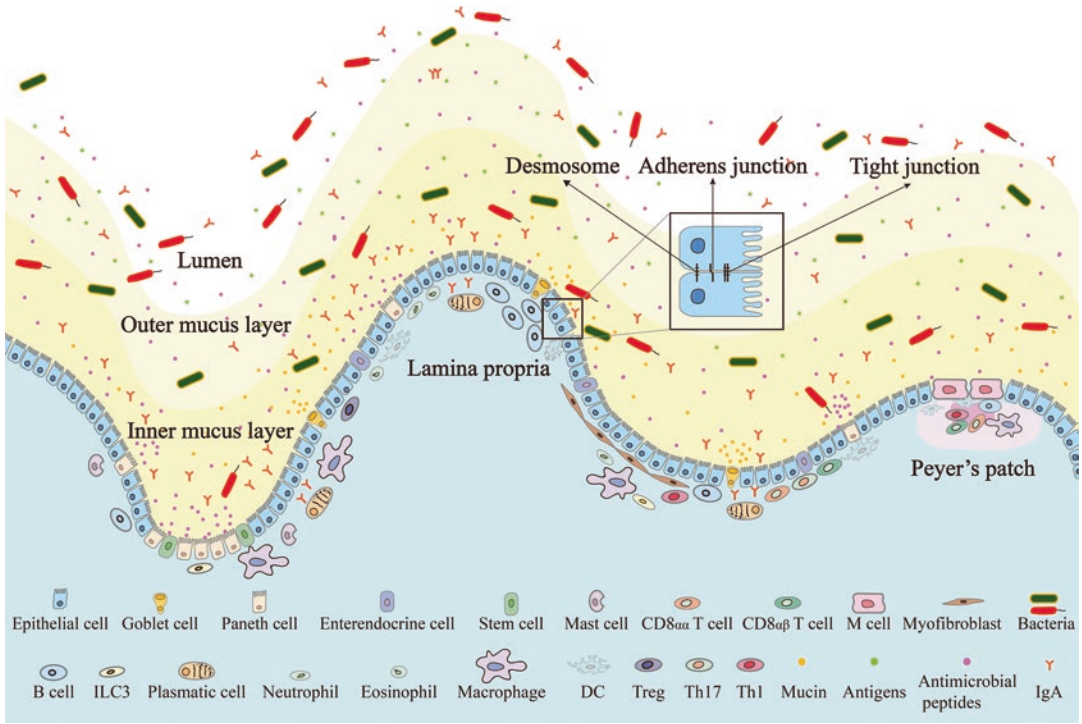

Fig. 8.1 The mucosal barrier defense system in the intestine. This defense system includes indigenous commensal microorganisms, epithelial layer, and the lamina propria. The epithelial layer consists of absorptive enterocytes, hormone-secreting enteroendocrine cells, mucin-secreting goblet cells, antimicrobial-secreting Paneth cells, intraepithelial lymphocytes (IELs), microfold cells, and dendritic cells. The lamina propria harbors various immune cells [e.g., dendritic cells (DC), neutrophils, macrophages, B cells, and T cells], fibroblasts, and blood vessels. ILC3 = group 3 innate lymphoid cell

nal immune response through its regulation of intestinal or systemic innate and adaptive immunities (Honda and Littman 2016; Thaiss et al. 2016), as well as by direct effects on pathogens via colonization resistance (Endt et al. 2010; Seekatz and Young 2014). For example, infection by *Clostridium difficile*, which is the leading health care-associated illness, usually follows the disruption of the indigenous gut microbiota after antibiotic treatment, leading to the loss of colonization resistance against the pathogen (Britton and Young 2014; Seekatz and Young 2014; Theriot et al. 2014). A successful treatment strategy for *C. difficile* infection is fecal microbiota transplantation from healthy individuals, which can recover the gut microbiome after transplantation (Fuentes et al. 2014; Seekatz et al. 2014).

Dietary AAs regulate the diversity, composition and metabolism of intestinal microbiota (Dai et al. 2011, 2015). For example, arginine decreases the net utilization of lysine, threonine, isoleucine, leucine, glycine and alanine by jejunal or ileal mixed bacteria (Dai et al. 2012). Arginine supplementation shifts the population of microbes in the jejunum and ileum of mice to favor the growth of *Bacteroidetes* by decreasing the number of *Firmicutes*, but increasing the abundance of *Bacteroidetes* (Ren et al. 2014a). Arginine also enhances the abundance of *Lactobacillus* in the jejunum and the abundance of *Streptococcus* in the ileum (Ren et al. 2014a). Thus, feeding *Lactobacillus reuteri* DSM 17938 to newborn mice increased the concentration of beneficial AAs and their metabolites in the large intestine, while regulating gut microbiota and immune responses (Liu et al. 2019b). In addition, dietary supplementation with glutamine to mice decreases the abundance of *Firmicutes* in their jejunum and

ileum, while increasing the abundance of *Streptococcus* and *Bifidobacterium* in their jejunum (Ren et al. 2014b). Furthermore, adding proline to the diet of Huanjiang mini-pigs decreases the amounts of *Klebsiella pneumoniae, Peptostreptococcus productus, Pseudomonas,* and *Veillonella* spp. in distal colonic contents (Ji et al. 2018). Likewise, dietary supplementation with gamma-aminobutyric acid (GABA) regulates the community richness and diversity of the ileal microbiota, as well as the abundances of the dominant microbial populations in weaned piglets (Chen et al. 2019b). Interestingly, dietary lysine restriction decreases the bacterial diversity and increases the abundance of *Actinobacteria*, *Saccharibacteria*, and *Synergistetes* in the intestine at the phylum level, as well as the abundances of *Moraxellaceae*, *Halomonadaceae*, *Shewanellaceae*, *Corynebacteriaceae*, *Bacillaceae*, *Comamonadaceae*, *Microbacteriaceae*, *Caulobacteraceae*, and *Synergistaceae* in the intestine at the family level (Yin et al. 2017).

The exact mechanisms whereby AAs modulate intestinal microbiota need further investigation. It is possible that AA supplementation or restriction alters the intestinal microenvironment, and then influences the composition and function of the intestinal microbiota. Notably, beneficial effects of AAs on gut health are associated with similar changes in the intestinal microbiota, but some AAs exert specific effects. Also, the influences of AAs on the intestinal microbiota depend on their supplemental dosages. For example, dietary supplementation with 0.5 and 1% aspartate to mice reduces the ratio of *Firmicutes* to *Bacteroidetes* in the ileum and feces, but dietary supplementation with 2% aspartate increases this ratio in the feces (Bin et al. 2017).

Results of our recent studies indicate that arginine or glutamine supplementation promotes the activation of intestinal innate immunity, including expression of factors (e.g., toll-like receptors) and activation of signaling pathways [e.g., mitogen-activated protein kinase (MAPK)] associated with intestinal innate immunity (Ren et al. 2014a, b). Thus, dietary supplementation with arginine or glutamine enhances the ability of the host to clear infections by pathogens (e.g., porcine circovirus type 2 and *Pasteurella multocida*) (Chen et al. 2014; Ren et al. 2012, 2013a, b, c, d), especially intestinal pathogens (e.g. enterotoxigenic *Escherichia coli*) (Liu et al. 2017a). However, whether arginine or glutamine promotes the clearance of these pathogens in the host through the intestinal microbiota remains to be explored.

Intestinal microbiota also affects the host AA metabolism and, therefore, the defensive responses. For example, the intestinal microbiota (*Clostridium sporogenes*) uses aromatic AAs (tryptophan, phenylalanine and tyrosine) as substrates to produce metabolites (e.g., indolepropionic acid), which in turn affect intestinal permeability and systemic immunity (Dodd et al. 2017). The enrichment of the intestinal microbiota that synthesizes the branched-chain amino acids (BCAA), such as *Prevotella copri* and *Bacteroides vulgatus*, and that have a low capacity to take up BCAAs, are associated with high concentrations of BCAA in serum (Pedersen et al. 2016). Indeed, the levels of AAs in the ileum differ markedly between conventionally reared and germ-free mice, indicating that the gut microbiota greatly affects the metabolism of AAs in the ileum (Mardinoglu et al. 2015). Those AAs include arginine, asparagine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and glutamine (Mardinoglu et al. 2015). It is unknown whether this alteration in AA metabolism is associated with the abnormities of intestinal immunity in germ-free mice, such as Paneth cell dysfunction (Zhang et al. 2015). During enterotoxigenic *Escherichia coli* infection, hosts (i.e., piglets and mice) experience remarkable alterations in the intestinal microbiota, especially increases in the abundance of *Lactococcus lactis* subsp. (Ren et al. 2016d). *Lactococcus lactis* subsp. regulates the host immune responses against enterotoxigenic *Escherichia coli* infection through producing GABA, which promotes intestinal expression of IL-17 to activate the mechanistic target of rapamycin complex 1 (mTORC1)-ribosomal protein S6 kinase 1 (S6K1) signaling (Fig. 8.2) (Ren et al. 2016d). Besides glycine and the L-isoform of AAs, the mouse intestine contains high levels of free D-AAs derived from the microbiota (Kepert et al. 2017; Sasabe et al. 2016). Interestingly, the intesti-

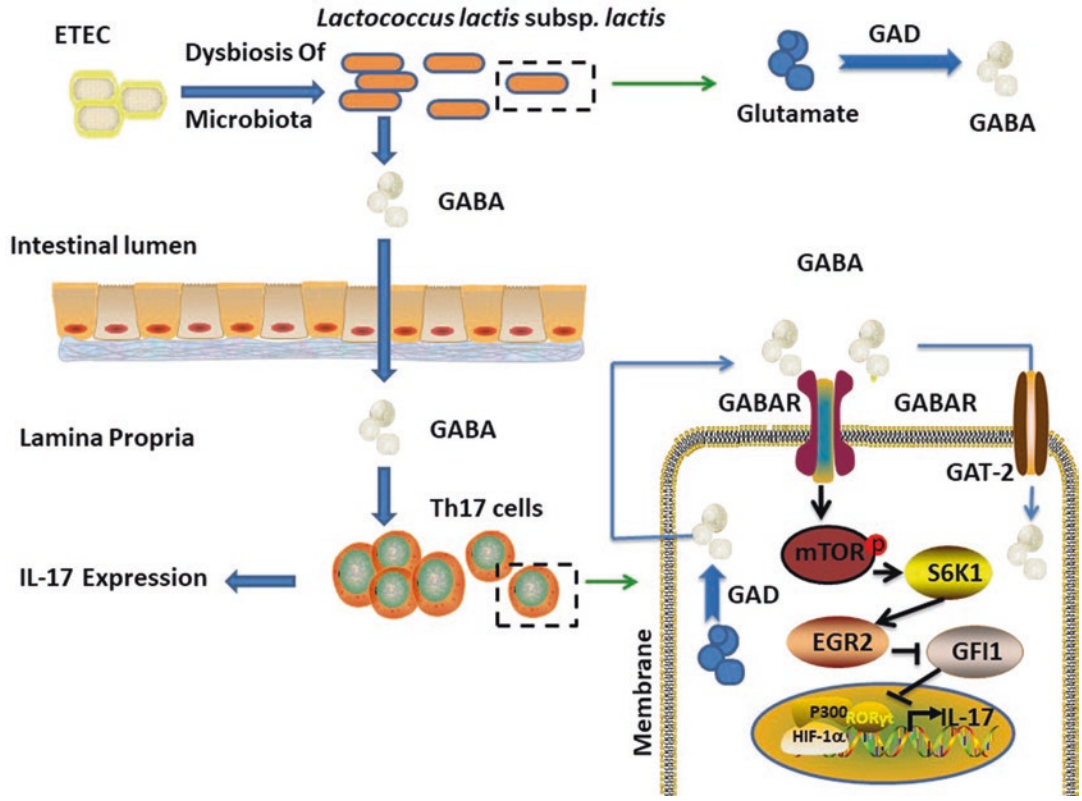

Fig. 8.2 γ-Aminobutyrate (GABA) mediates intestinal interleukin-17 expression during infection by enterotoxigenic *Escherichia coli* (ETEC). During ETEC infection, the pathogen induces dysbiosis in the gut microbiota, increasing *Lactococcus lactis subsp*. The *Lactococcus lactis subsp.* produces GABA from glutamate through the action of glutamate decarboxylase (GAD). GABA is sensed by Th17 cells through GABA receptors (GABAR), leading to the activation of the mTOR pathway. The mTOR signaling promotes IL-17 expression during infection through the mTOR-S6K1-EGR2-GFI1 pathway. GABA transporter 2 is negatively associated with Th17 response during intestinal infection by terminating the GABA signaling through the translocation of GABA from the extracellular to the intracellular space

nal microbiota stimulates the release of D-amino acid oxidase (DAO) from intestinal epithelial cells (including goblet cells) into the intestinal lumen, resulting in the oxidative deamination of intestinal D-AAs to yield a potent antimicrobial product, H_2O_2, thereby protecting the mucosal surface in the small intestine from the cholera pathogen (Sasabe et al. 2016). DAO has also been shown to modify the composition of the microbiota and production of intestinal sIgA (Sasabe et al. 2016). This illustrates the importance of D-AAs in nutrition and metabolism. Collectively, there is significant crosstalk between host AAs and the intestinal microbiota, and this interplay regulates the intestinal defensive responses and the progression of intestinal infection.

8.3 Amino Acids and Intestinal Epithelial Cells

Besides the absorption of nutrients (including AAs, glucose, fatty acids, and electrolytes), intestinal epithelial cells (generated from intestinal epithelial stem cells) represent an effective barrier ling the gastrointestinal mucosal surface, and regulate the functions of intestinal immune cells as well as intestinal homeostatic and inflam-

matory responses (Nowarski et al. 2017). For example, the villous epithelial cells that express the neonatal Fc receptor (FcRn) play a role in binding intestinal antigens (McDole et al. 2012; Schulz and Pabst 2013). FcRn on villus epithelial cells aids in the secretion of IgG across the intestinal epithelium into the lumen, and also contributes to the uptake of intestinal antigens from the lumen through the IgG-dependent process (Yoshida et al. 2004, 2006). Also, the expression of *Ifnlr1* [the receptor for interferon (IFN)-λ] on intestinal epithelial cells in the small intestine and colon is critical for enteric IFN-λ antiviral activity in mice (Baldridge et al. 2017). Importantly, *Ifnlr1* expression in intestinal epithelial cells affects the efficacy of IFN-λ in resolving persistent murine norovirus infection, and is necessary for the sterilizing innate immune effects of IFN-λ (Baldridge et al. 2017). Although p40 (a *Lactobacillus rhamnosus* GG-derived protein) treatment directly on B cells shows little effect on IgA production, p40 promotes the expression of a proliferation-inducing ligand (APRIL) in intestinal epithelial cells, resulting in an increase in fecal IgA levels, as well as IgA^+B220^+, IgA^+CD19^+, and IgA^+ plasma cells in the lamina propria of mice (Wang et al. 2017). Collectively, intestinal epithelial cells are closely associated with intestinal immunity responses.

It is well known that AAs, such as glutamate, cysteine, glutamine and glycine, promote protein synthesis in intestinal epithelial cells and their growth via various cellular signaling, such as the mTOR signaling (He et al. 2016; Honda and Littman 2016; Wang et al. 2014a, 2016; Ye et al. 2016), as well as nutrient metabolism, glutathione synthesis, and ATP production (Li et al. 2020). For example, arginine enhances DNA synthesis, cell-cycle progression, and mitochondrial bioenergetics in intestinal epithelial cells through mechanisms involving activation of the phosphatidylinositol 3′-kinase (PI3K)-protein kinase B (Akt pathway) (Tan et al. 2015). Given the importance of AAs in these physiological processes, we surmise that AAs may affect intestinal defensive responses by regulating the expression and secretion of immune regulators in intestinal epithelial cells. For example, BCAA stimulate the expression of β-defensin from porcine intestinal epithelial cells possibly through activation of the sirtuin-1(Sirt1)/extracellular regulated protein kinases (ERK) signaling pathway (Ren et al. 2016a). In addition, tryptophan inhibits the secretion of interleukin (IL)-8 in intestinal epithelial cells after tumor necrosis factor (TNF)-α challenge through the calcium-sensing receptor (Mine and Zhang 2015). Glycine attenuates the production of reactive oxygen species (ROS) in intestinal epithelial cells via promoting the synthesis of glutathione and expression of glycine transporter 1, while reducing the activation of the MAPK signaling pathway (Wang et al. 2014a). Amino acids also regulate the function of intestinal epithelial cells and the intestinal immunity. For example, AA starvation in intestinal epithelial cells induces autophagy responses in intestinal epithelial cells, resulting in lower levels of ROS and IL-1beta as well as a reduction in the abundance of IL-17A-producing $CD4^+$ T cells (Ravindran et al. 2016). Collectively, epithelial cells are involved in intestinal immune responses, such as antigen recognition, IgA production, and the killing of pathogens. Some AAs (e.g., arginine, BCAA and glycine) regulate protein synthesis in intestinal epithelial cells, their proliferation and migration, as well as the generation and secretion of immune regulators by the cells.

8.4 Amino Acids and Intercellular Junction

Between intestinal epithelial cells, there are intercellular junctions that include an apical tight junction (TJ), subjacent adheren junction (AJ), and desmosomes, controlling the movement of fluids and solutes in the paracellular space and the establishment of cell polarity (Luissint et al. 2016; Tsukita et al. 2001). Tight junctions reside include claudins, TJ-associated MARVEL domain-containing proteins (TAMPs, including occludin, MARVELD2, and MARVELD3), and members of the cortical thymocyte marker in the Xenopus family, such as junctional adhesion molecules (Luissint et al. 2016; Raleigh et al. 2010). The AJ is an ancient junctional complex that initiates and maintains epithelial cell-cell

contacts, while the desmosomes provides mechanical strength to the epithelium. The key transmembrane protein in the epithelial AJ is E-cadherin, while the desmosomes include desmoglein and desmocollin proteins (Green and Simpson 2007; Ivanov and Naydenov 2013). The maintenance of the intestinal epithelial barrier is dependent on the crosstalk among TJs, AJs, and desmosomes (Luissint et al. 2016). A functional intestinal epithelium allows for selective absorption of nutrients, while restricting the passage of pathogens and food-borne antigens. However, various intestinal pathogens have been reported to target the intestinal epithelial barrier to induce disassembly and barrier defects. For example, the enterotoxin produced by *Clostridium perfringens* has been reported to bind claudins 3, 4, 6, 7, 8, 9, and 14, resulting in their internalization from the TJ and therefore compromising mucosal barrier function (Fernandez Miyakawa et al. 2005; Saitoh et al. 2015; Veshnyakova et al. 2010).

Dietary AAs are important regulators of intercellular function, especially the expression and abundance of TJs. This notion is supported results from both *in vitro* and *in vivo* experiments. For example, tryptophan enhances the abundances of occludin, claudin-4, zonula occludens (ZO)-1 and 2 in intestinal porcine epithelial cells (Wang et al. 2015a). Similarly, glutamine decreases the TJ permeability, but increases the monolayer transepithelial electrical resistance (TEER), the abundances of transmembrane proteins (including occludin, claudin-4, ZO-1, ZO-2, and ZO-3) through activation of the calcium/calmodulin-dependent kinase 2 (CaMKK2)-AMP-activated protein kinase (AMPK) signaling (Jiao et al. 2015; Wang et al. 2016). Subsequent investigations with piglets also demonstrate the positive influence of physiological levels of AAs on the expression of TJ proteins. Specifically, dietary supplementation with glutamine to weanling piglets augments the abundances of occludin, claudin-1, ZO-2, and ZO-3 proteins in the jejunum (Wang et al. 2015b). Besides glutamine, dietary supplementation with putrescine or proline to neonatal piglets between day 1 of age and weaning at 14 day of age increases the abundances of ZO-1, occludin, and claudin-3 proteins in the jejunum (Wang et al. 2015c). Similarly, studies with post-weaning pigs have shown that dietary supplementation with 1% glutamine (Wu et al. 1996), 1% proline (Wu et al. 2011), or 1-2% glycine (Wang et al. 2014b) ameliorated intestinal atrophy and improved their growth performance, whereas dietary supplementation with 0.2% putrescine dihydrochloride improved intestinal integrity and decreased the incidence of diarrhea (Liu et al. 2019a). Note that glutamine, glycine and proline are highly abundant in animal-source proteins such as meat & bone meal, poultry by-products, and chicken visceral digest (Wu and Li 2020), whereas the content of glycine and proline is relatively low in all plant-source proteins (Hou et al. 2019).

Animals are frequently exposed to stressful conditions in their life times. Importantly, AAs are beneficial for maintaining the adequate expression of intestinal TJ proteins in subjects with various intestinal diseases, such as intestinal inflammation that is associated with the defect of TJ functions. In the dextran sulfate sodium (DSS)-induced colitic mouse model, which is similar to human ulcerative colitis, dietary supplementation with arginine or glutamine increases the abundance of the claudin-1 protein in the colon (Ren et al. 2014c). Likewise, glutamine administration increases the abundance of the ZO-1 protein in the small-intestinal mucosa of DSS-treated mice (Pai et al. 2014). Similarly, in rats with methotrexate-induced mucositis, glutamine or arginine supplementation enhances the jejunal abundances of claudin-1, occludin and ZO-1 proteins through ERK and NF-κB pathways (Beutheu et al. 2014). In addition to intestinal inflammation, AAs are also essential for the homeostasis of TJ proteins in other situations. For example, although a western-style high-fat diet lowers the levels of occludin and ZO-1 proteins in the upper part of the mouse small intestine, oral administration of arginine restores the abundances of occludin and ZO-1 proteins (Sellmann et al. 2017a). Liang et al. (2018) reported that dietary supplementation with 0.2% tryptophan to weanling pigs increased the abundances of ZO-1 and occludin proteins in the colon. Furthermore, dietary supplementation

with 0.2% and 0.4% tryptophan to weanling pigs augmented the abundances of the jejunal ZO-1, ZO-3 and claudin proteins in a dose-dependent manner, whereas dietary supplementation with 0.4% tryptophan also enhanced the abundance of the jejunal occludin protein (Liang et al. 2019). Thus, much evidence shows that intercellular junctions, including TJ, AJ and desmosomes, play a critical role in the hemostasis of the intestinal epithelium. Dietary AAs are essential for the expression of the TJ proteins, especially in various intestinal diseases that are characterized by defects in the intestinal mucosal barrier (Table 8.1). However, it remains unknown how AAs affect the location of intestinal TJ proteins or the homeostasis of the intestinal AJ and desmosomes. This remains to be an active area of research in AA physiology and nutrition.

8.5 Amino Acids and Goblet Cells

In addition to enterocytes, the second subtype of cells in the intestinal epithelium is the mucus-producing goblet cells. Goblet cells are specialized secretory cells lining intestinal mucosal epithelia. The differentiation of goblet cells from intestinal epithelial stem cells is tightly regulated by the sterile α motif pointed domain epithelial specific transcription factor (*Spdef*), which responds to the downstream of both Notch and Wnt signaling. *Spdef*-null mice show a reduction in mature, differentiated goblet cells in the intestine, whereas overexpression of *Spdef* in the intestine displays an expansion of Muc2-expressing goblet cells at the expense of other intestinal cell types (Gregorieff et al. 2009; Noah et al. 2010). Goblet cells have critical roles in maintaining intestinal homeostasis through secreting a variety of factors, such as mucins and trefoil factors (Johansson and Hansson 2016; McCauley and Guasch 2015). The secretion of these factors from goblet cells depends on various stimuli, such as microbial factors, growth factors and inflammatory cytokines (Deplancke and Gaskins 2001; McCauley and Guasch 2015; Wlodarska et al. 2014), as well as the availability of threonine (a major AA in mucins; Wu 2018). These factors entrap external insults such as pathogens, toxins, and allergens, and prevent their translocation into the blood and other extra-intestinal tissues (Johansson and Hansson 2016). Besides the secretory function, goblet cells have recently been implicated as antigen-presenting cells because goblet cells in the small intestine present intestinal luminal antigens to the underlying dendritic cells so that dendritic cells can sense intestinal insults without a break in intestinal barrier integrity (Knoop et al. 2015; McDole et al. 2012).

Increasing evidence has shown that dietary AAs actively maintain the number of intestinal goblet cells and the expression of mucins in the intestine. For example, in healthy mice, dietary supplementation with 1.0% glutamine for 2 weeks promotes the expression of mucin-4 in the jejunum (Ren et al. 2014b). Similar observation has also been reported in various models of intestinal diseases. For example, in rats with DSS-induced colitis, dietary supplementation with a mixture of AAs (containing L-threonine, L-serine, L-proline, and L-cysteine) attenuates reductions in the number of Muc2-containing goblet cells in the intestinal epithelium of the ulcerated area and mucin production in the colon, while restoring the mucin AA composition and mucosal content (Faure et al. 2006). Likewise, in rats with experimental diversion colitis, glutamine supplementation increases the number of goblet cells in the colonic lamina propria (Pacheco et al. 2012). Also, in enterotoxigenic *Escherichia coli* (ETEC) infected mice, glutamine promotes the expression of mucin-2 in the jejunum (Xu et al. 2017), providing another line of evidence for a crucial role of the functional AA in gut integrity and function (Rhoads and Wu 2009).

Under certain experimental conditions, some AAs have little effect on or even reduce the number of intestinal goblet cells. For example, glutamine supplementation to weaning mice did not affect the number of goblet cells, or the expression of markers for goblet cells (Chen et al. 2018a). In male 50-day-old Wistar rats, dietary supplementation with 2.0% glutamine for 10 days

Table 8.1 Effects of amino acids on intestinal tight junction proteins

Amino acids	Models	Effect on TJs	References
Tryptophan	IPEC	Tryptophan increases the protein abundances of occludin, claudin-4, ZO-1 and ZO-2.	Wang et al. (2015a)
	Pigs	Tryptophan supplementation enhances the mRNA levels of claudin-3 and ZO-1.	Liu et al. (2017b)
Methionine	Rats	Methionine restriction increases the mRNA levels of claudin-3 and changes the posttranslational modification of occludin.	Ramalingam et al. (2010)
	Renal epithelial cells	Methionine restriction decreases the protein abundances of claudin-3 and 7, but dramatically increases the abundances of claudin-4 and 5.	Mullin et al. (2009)
Glycine	IPEC	Glycine supplementation enhances the protein abundances of claudin-3, claudin-7 and ZO-3.	Li et al. (2016)
Valine	Grass carp	Valine deficiency decreases mRNA levels of claudin-b, claudin-3, occludin and ZO-1, but increases the mRNA level of claudin-15.	Feng et al. (2015b)
Phenylalanine	Grass carp	Phenylalanine supplementation increases the mRNA levels of ZO-1, occludin and claudin-c.	Feng et al. (2015a)
Leucine	Grass carp	Leucine supplementation increases the mRNA levels of occludin, ZO-1, claudin-b, claudin-3 and claudin-12.	Jiang et al. (2017b)
Isoleucine	Hen	Excess digestible isoleucine level does not change mRNA levels of claudin-1 and occludin.	Dong et al. (2016)
	Grass carp	Isoleucine deficiency down-regulates the mRNA levels of claudin-3, claudin-b, claudin-c, occludin and ZO-1, but up-regulates the mRNA level of claudin-12.	Feng et al. (2017)
Proline	Piglet	Proline increases the protein abundances of ZO-1, occludin and claudin-3.	Wang et al. (2015c)
Glutamine	IPEC	Glutamine increases the protein abundances of occludin, claudin-4, junction adhesion molecule (JAM)-A, ZO-1, ZO-2 and ZO-3.	Wang et al. (2016)
	Weanling piglet	Glutamine increases the protein abundances of occludin, claudin-1, ZO-2, and ZO-3.	Wang et al. (2015b)
	Caco-2 cells	Deprivation of glutamine decreases protein abundances of claudin-1, occludin and ZO-1.	Li et al. (2004)
Glutamate	IPEC	Glutamate enhances the mRNA and protein abundances of occludin, claudin-3, ZO-2 and ZO-3.	Jiao et al. (2015)
	Caco-2 cells	Glutamate supplementation increases the mRNA levels of ZO-1 and occludin during MTX treatment.	Beutheu et al. (2013)
	Carp	Glutamate supplementation increases mRNA levels of ZO-1, occludin, claudin-2, 3 and 7 during LPS challenge.	Jiang et al. (2017a)
Arginine	Caco-2 cells	Arginine supplementation increases the mRNA levels of ZO-1 and occludin during MTX treatment.	Beutheu et al. (2013)
	Grass carp	Arginine supplementation enhances the mRNA levels of occludin, claudin-3 and claudin-c.	Chen et al. (2019a)
Threonine	Broiler chickens	Threonine administration increases the mRNA levels of claudin-3 and ZO-1 during LPS challenge.	Chen et al. (2018b)
Histidine	Grass carp	Histidine deficiency down-regulates the mRNA levels of claudin-b, claudin-c, claudin-3, claudin-12, claudin-15, occludin and ZO-1.	Jiang et al. (2016)
Citrulline	Mice	Citrulline increases the protein abundances of occludin and ZO-1.	Sellmann et al. (2017b)

was reported to decrease the numbers of goblet cells in the villi and crypt of the jejunum or ileum (Martins et al. 2016). Similarly, glutamine supplementation reduced the number of goblet cells in the villi and crypt of jejunum or ileum in rats with Walker-256 tumor (Martins et al. 2016). However, the provision of glutamine from the basal diet was not known in all these studies. In weaned piglets, tryptophan supplementation had little effect on the numbers of goblet cells in the duodenum, jejunum and ileum (Tossou et al. 2016). Similarly, threonine supplementation did not influence the numbers of goblet cells in the jejunum and colon or the amounts of mucins in the scrapings of the jejunum and colon in weaning piglets (Trevisi et al. 2015). The possible reasons for these discrepancies include animal models, intakes of the AAs from the basal diets, supplemental dosages of the AAs, and the methods used for the analysis of goblet cells. Thus, the influences of AA in intestinal goblet cells need further investigation. It is interesting to determine whether specific AAs (e.g., glutamine, arginine and glycine) regulates the differentiation of intestinal goblet cells from intestinal epithelial stem cells.

8.6 Amino Acids and Paneth Cells

With the lineage of secretory cells from intestinal epithelial stem cells, Paneth cells produce antimicrobial peptides, which are rich in proline (Hou et al. 2017). Various cellular signaling pathways affect the differentiation of Paneth cells, such as Notch, PKC λ/ι and mTORC1 (Heuberger et al. 2014; Nakanishi et al. 2016; Zhou et al. 2015). Unlike the enterocytes, Paneth cells reside at the base of the small intestinal crypts of Lieberkühn, where epithelial stem cells are also present.

Paneth cells secrete a wide variety of peptides and proteins, such as lysozyme, α-defensin peptides and secretory phospholipase A2 isotype II (Clevers and Bevins 2013; Porter et al. 2002; Salzman and Bevins 2013). Most of these peptides and proteins have antimicrobial effects, which target microorganisms, including the resident microbiota of the small intestine and the intruding pathogens that can potentially penetrate the mucus layer to invade the crypt or other parts of the intestinal epithelium (Ayabe et al. 2000; Bevins and Salzman 2011). Thus, Paneth cells help protect the gut from pathogenic microbes and shape the composition of the intestinal resident microbiota (Brandl et al. 2007; Salzman et al. 2010; Veshnyakova et al. 2010).

Paneth cells also secrete ligands that provide trophic support for the adjacent epithelial stem cells (Sato et al. 2011). These peptides and proteins are stored in the large secretory granules of Paneth cells and secreted into the crypt lumen via mechanisms mediated by KCa3.1 calcium-activated potassium channels in response to a variety of stimuli, including bacterial products (Ayabe et al. 2000, 2002).

Dietary AAs have been reported to regulate the production of antimicrobial peptides by Paneth cells. For example, arginine supplementation upregulates the expression of *cryptdins 1*, *4*, and *5*, cryptdin-related sequence 1c (*Crs1c*), and RNase angiogenin 4 (*Ang4*) in the jejunum and ileum (Ren et al. 2014a). Similarly, glutamine supplementation increases the mRNA levels for *cryptdins 1*, *4*, and *5* in the jejunum, *cryptdins 4* in the ileum, and *Reg3g* in the colon (Ren et al. 2014b). In ETEC-infected mice, arginine or glutamine supplementation also promotes the expression of the *Crs1c* and *Reg3g* genes (Liu et al. 2017a). Although these results indicate the beneficial function of arginine or glutamine in Paneth cells, the underlying mechanisms are largely unknown. It remains to be determined whether other functional AAs regulate the differentiation of Paneth cells or the expression of antimicrobial peptides in the cells. Collectively, arginine or glutamine can modulate the synthesis of antimicrobial peptides in Paneth cells. However, the roles of other AAs in the secretion

of intestinal antimicrobial peptides and the differentiation of Paneth cells remain to be explored.

8.7 Amino Acids and Intestinal Immune Cells

There are various types of immune cells in the intestine, including IELs, microfold cells, dendritic cells, macrophages, B cells, and T cells (Fig. 8.1). The intestine has now been characterized as the largest lymphoid organ in humans and other mammals. The intricate intestinal immune system consists of the outer epithelial layer and the inner lamina propria. The components of the outer section include IELs, the dendritic cell extensions and microfold cells. Intraepithelial lymphocytes are an important line of the first defense that maintains the integrity of intestinal epithelial cells, and dendritic cells help to determine the type of immune response as needed by presenting luminal antigens. In pigs, IELs respond well to T-cell mitogens after weaning but not during the preweaning period (Wu 1996). Microfold cells also mediate the transcytosis of antigens across the epithelium. The inner section of the intestinal defense locates below the IELs, and includes dendritic cells, neutrophils, macrophages, immunoglobulin (Ig) A-producing B cells, natural killer (NK) cells, NK T-cells, conventional T-cells, and T-regulatory cells.

The numbers of macrophages, T cells, and B cells in the intestinal mucosa are greater in weanling mammals (e.g., pigs) than in preweaning ones (Wu 1995). These immune cells initiate inflammation and injury in the gut. Available evidence shows that AAs are important regulators of the activation and function of intestinal immune cells. For example, glutamine promotes the secretion of IgA and increases the abundance of IgA-producing B cells in the intestine (Ren et al. 2016b; Wu et al. 2016). Glutamine also highly shapes the polarization of macrophages through mechanisms, including glutamine-UDP-*N*-acetylglucosamine pathway, glutamine-derivedα-ketoglutarate via glutaminolysis, and glutamine-dependent anerplerosis or the GABA shunt (Ren et al. 2019a; Xia et al. 2019). Dietary deficiency of AAs significantly reduces the number of $F4/80^+CD11b^+$ macrophages and the number of $IL\text{-}10^+F4/80^+CD11b^+$ macrophages in the mouse small intestine (Ochi et al. 2016). The influence of dietary AAs on small-intestinal macrophages may depend on mTOR signaling because an inhibition of this signaling by rapamycin also reduces the number of $IL\text{-}10^+F4/80^+CD11b^+$ macrophages in the mouse small intestine (Ochi et al. 2016).

Considering the importance of AAs in T cell fate decision (Ren et al. 2016c, 2017a, b), it is not surprising that AAs regulate intestinal T cell response. For example, during ETEC infection, intestinal GABA promotes the expression of IL-17 in the jejunum of both mice and piglets (Ren et al. 2016d, 2019b). In addition to conventional T-cells, AAs also modulate the intestinal un-conventional T-cell response. For example, in the DSS-treated mice, glutamine administration increases the proportion of small-intestinal IEL γδ-T cells but decreases the expression of genes responsible for immunomodulation in IEL γδ-T cells, such as *Ifn-γ*, *Tnf-α* and *Il-17* (Pai et al. 2014). Similarly, glutamine decreases the percentage of IEL γδ-T cells, and regulates the mRNA expression of *Bcl-xl*, *Il-7 receptor* and *Reg3g* in IEL γδ-T cells in mice with ischemia/reperfusion injury (Pai et al. 2015). Furthermore, dietary supplementation with L-tryptophan (0.1 g/kg body weight per day) to mice with DSS-induced inflammation reduced the abundances of macrophages and neutrophils in the colon and improved colonic immune responses partly through attenuating the activation of toll-like receptor 4 (TLR4)-STAT3 signaling and nucleus p-65 (Wang et al. 2020). Thus, dietary AAs play an important role in the activation and function of intestinal immune cells (e.g., IgA-producing B cells, macrophages and T cells, Table 8.2). However, the influences of AAs on the number and function of other types of intestinal immune cells, such as M cell, dendritic cells and neutrophils, need further investigation. Besides the mTOR signaling, whether AAs affects the fate of intestinal immune cells through other cellular signaling molecules (such as nitric oxide, kynurenine, glycine, glutamate and hydroxyproline) remain to be determined (Hou and Wu 2018; Li and Wu 2018; Wang et al. 2013, 2020; Wu et al. 2019b).

Table 8.2 Effects of amino acids on intestinal immunity

Amino acids	Models	Effect on intestinal immunity	References
Tryptophan	Acetic acid-treated mice	Tryptophan supplementation inhibits the colonic mRNA expression of IL-22.	Chen et al. (2018a)
	DSS- treated mice	Tryptophan supplementation reduces the colonic mRNA levels of IL-6, TNF-α, IL-1β, CCL2, CXCL1 and CXCL2.	Islam et al. (2017)
Methionine	Chemotherapy-induced intestinal mucositis rats	Methionine supplementation increases the intestinal mRNA levels of IL-10.	Wu et al. (2019a)
Glycine	Rodent postoperative inflammatory ileus	Glycine treatment reduces the mRNA levels of IL-6 and TNF-α in the rat small intestinal muscularis.	Stoffels et al. (2011)
Valine	Normal grass carp	Valine deficiency down-regulates mRNA levels of IL-10 and TGF-β1, but up-regulates the mRNA levels of TNF-α and IL-8 in the small intestine.	Luo et al. (2014)
Phenylalanine	Normal grass carp	Phenylalanine supplementation increases the mRNA levels of IL-10 and TGF-β1 in the intestine.	Feng et al. (2015a)
Leucine	LPS-treated chicken embryos	Leucine supplementation decreases IgA production and mRNA level of IL-6 in small intestine.	Liu et al. (2018)
	Normal grass carp	Leucine supplementation down-regulates the mRNA levels of TNF-α and IL-8 in the mid and distal intestine.	Jiang et al. (2015)
Serine	Early-weaned piglets	Serine supplementation decreases both cytokine secretion and mRNA levels of IL-1β, IL-6, IL-8 and TNF-α in the small intestine.	Zhou et al. (2018)
	LPS-treated mice	Serine treatment reduces the mRNA levels of IL-1β, TNF-α, IL-6, IL-8 and IL-10 in the ileum.	Zhou et al. (2017)
Cysteine	LPS-treated piglets	Cysteine supplementation down-regulates the mRNA levels of TNF-α, IL-6 and IL-8 in the jejunum and ileum.	Song et al. (2016)
	DSS-treated piglets	Cysteine supplementation reduces the colonic mRNA levels of TNF-α, IL-6, IL-12p40 and IL-1β.	Kim et al. (2009)
Asparagine	LPS-treated piglets	Asparagine supplementation down-regulates the intestinal TNF-α secretion.	Chen et al. (2016)
Glutamine	Normal mouse	Glutamine supplementation increases ileal mRNA levels of IL-5, IL-6, IL-13 and TGF-β.	Wu et al. (2016)
	DSS-treated mouse	Glutamine administration increases the proportion of small-intestinal IEL γδ-T cells but decreases the mRNA levels of IFN-γ, TNF-α and IL-17 in IEL γδ-T cells.	Pai et al. (2014)
	Normal mouse	Glutamine supplementation enhances mRNA levels for IL-1β, IL-17 and TNF-α in the ileum.	Ren et al. (2014b)
	Soybean meal-induced enteritis turbot	Glutamine decreases the infiltration of leucocytes in the lamina propria and submucosa, as well as the mRNA levels of IL-8, TNF-α and TGF-β in the intestine.	Gu et al. (2017)
Threonine	LPS-treated chicken	Threonine administration reduces mRNA levels of the jejunal IFN- γ and ileal IL-1β.	Chen et al. (2018c)
	IUGR weanling piglets	Threonine supplementation reduces the ileal mRNA level of TNF-α, and increases the production of Muc2 and SIgA, as well as the density of goblet cells.	Zhang et al. (2019)
Aspartate	Normal mouse	Aspartate supplementation decreases the ileal mRNA levels of IL-17, IFN-γ and Muc2.	Bin et al. (2017)
Glutamate	LPS-treated Jian carp	Glutamate treatment suppresses the mRNA levels of IL-1β, IL-8 and TNF-α, but enhances the mRNA levels of IL-10 in the intestine.	Jiang et al. (2017a)

(continued)

Table 8.2 (continued)

Amino acids	Models	Effect on intestinal immunity	References
Arginine	*Clostridium perfringens* infected chickens	Arginine supplementation elevates jejunal mRNA levels of IFN-γ, IL-10 and NOD1.	Zhang et al. (2017)
	Normal mouse	Arginine supplementation increases the ileal mRNA levels of IL-1β, TNF-α and IFN-γ, and promotes the production of SIgA, mucins, and Paneth antimicrobials in the jejunum and ileum.	Ren et al. (2014a)
	LPS-treated weaned pigs	Arginine administration reduces the mRNA levels of jejunal IL-6, and jejunal and ileal TNF-α.	Liu et al. (2008)
γ-aminobutyric acid	Normal weaned piglets	γ-aminobutyric acid supplementation inhibits the intestinal mRNA levels of IL-22, IL-1, IL-18 and Muc1, but increases the mRNA levels of IFN-γ, IL-4 and IL-10.	Chen et al. (2019b)
	ETEC infected mouse or piglets	Intestinal γ-aminobutyric acid promotes the mRNA level of IL-17 in the jejunum.	Ren et al. (2016d)

8.8 Conclusion

The intestine interacts with a diverse community of antigens and bacteria, and has evolved with a complex defense system, including the indigenous intestinal microbiota, epithelial layer and lamina propria. Dietary intakes of AAs profoundly affect this defense system that involves not only luminal microbes but also intestinal epithelial cells, TJs, globet cells, Paneth cells and immune cells (e.g., macrophages, B cells and T cells). It is imperative to explore the roles of AAs on the function of other components of the intestinal defense system, such as tuft cells, enteroendocrine cells and intestinal innate lymphoid cells. Through modulation of the intestine immune and anti-inflammatory systems, AAs can control the progression of various intestinal diseases, such as intestinal infection and intestinal colitis. However, we eagerly await further investigations of the new roles of AAs in intestinal physiology and pathology, and more evidence about the benefits of manipulating AA metabolism for mitigating intestinal diseases. In practice, adequate intakes of dietary AAs, particularly functional AAs (Wu 2010), are crucial for maintaining the integrity and function of the intestine and the whole-body in humans and other animals.

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Conflict of Interest The authors declare that they have no conflict of interest.

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