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ORIGINAL ARTICLE

Bioactive dietary peptides and amino acids in inflammatory bowel disease

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Abstract Inflammatory bowel disease (IBD), most commonly ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammation of the gastrointestinal tract. Patients affected with IBD experience symptoms including abdominal pain, persistent diarrhea, rectal bleeding, and weight loss. There is no cure for IBD; thus treatments typically focus on preventing complications, inducing and maintaining remission, and improving quality of life. During IBD, dysregulation of the intestinal immune system leads to increased production of pro-inflammatory cytokines, such as TNF-α and IL-6, and recruitment of activated immune cells to the intestine, causing tissue damage and perpetuating the inflammatory response. Recent biological therapies targeting specific inflammatory cytokines or pathways, in particular TNF-α, have shown promise, but not all patients respond to treatment, and some individuals become intolerant to treatment over time. Dietary peptides and amino acids (AAs) have been shown to modulate intestinal immune functions and influence inflammatory responses, and may be useful as alternative or ancillary treatments in IBD. This review focuses on dietary interventions for IBD treatment, in particular the role of dietary peptides and AAs in reducing inflammation, oxidative stress, and apoptosis in the gut, as well as recent advances in the cellular mechanisms responsible for their anti-inflammatory activity.

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Abbreviations

γ-EC	γ-Glutamylcysteine
γ-EV	γ-Glutamylvaline
AA	Amino acids
Apolipoprotein L6	APOL6
CaSR	Extracellular calcium-sensing receptor
IBD	Inflammatory bowel disease
UC	Ulcerative colitis
CD	Crohn's disease
DC	Dendritic cells
IECs	Intestinal epithelial cells
GALT	Gut-associated lymphoid tissue
IL	Interleukin
ROS	Reactive oxygen species
Th	T helper
TLRs	Toll-like receptors
DSS	Dextran sodium sulfate
TNBS	2,4,6-Trinitrobenzene sulfonic acid
TNF-α	Tumor necrosis factor-alpha

Introduction

Inflammation is a complex of biological reactions generated by the innate immune system in response to tissue injury, infection, and irritation, which is a normal defensive activity of the body that eliminates pathogens. The process of inflammation is initiated by the migration of immune cells through the blood to the site of injury (Pan et al. 2010). Subsequently, inflammatory mediators, including reactive oxygen species (ROS) and pro-inflammatory

cytokines, are released from immune cells, resulting in the development of clinical symptoms including: redness, swelling, pain and fever, and subsequent activation of adaptive immune responses. A typical chronic inflammatory disease that occurs in the gastrointestinal (GI) tract is inflammatory bowel disease (IBD). The GI tract is the primary organ that directly interacts with dietary compounds and provides a complex barrier system against foodborne pathogens and toxic agents, while allowing nutrient absorption and water transportation into the body. Development of chronic inflammation in the GI tract leads to the disruption of normal absorption and digestion processes and increases the risk of developing disease-related complications. The excessive production of inflammatory mediators in the gut induces the extensive infiltration of immune and inflammatory cells into the intestinal mucosa that amplifies the inflammatory responses. During the persistent inflammatory phase, the increasing production of pro-inflammatory cytokines and ROS result from activation of signaling cascades, which in turn, contribute to the progression of immune responses and initiate the cycle of chronic pro-inflammatory processes. Subsequently, impaired function of the intestinal mucosal barrier, permanent tissue damage, and associated abnormal cell proliferation eventually develop from the above process, which are characteristic of IBD. IBD, which includes ulcerative colitis (UC) and Crohn's disease (CD), is a result of a chronic and relapsing inflammation of the GI tract, induced by complex of genetic, immunological, environmental, and lifestyle factors (Sartor 2007). The immunogenic mechanisms in IBD are complex due to the two subtypes (UC and CD) exhibiting different immune responses to various infections; therefore, effective strategies to cure IBD are limited. Conventional treatments, such as immunosuppressive drugs and biological therapies which target specific inflammatory cytokines or pathways, have been shown to have either low efficacy or adverse side effects (de Silva et al. 2010). Consequently, alternative strategies need to be developed to restore intestinal mucosal homeostasis and treat IBD. Current studies on nutrient supplementation have suggested that the essential nutrients or functional dietary components derived from daily foods have the potential to ameliorate gut inflammation and restore redox balance in the gut. While nutrient supplementation is considered to be safe, the possible interaction of the constituents of supplementation with the intestinal mucosal system has not been well studied.

The study of immuno-nutrition has been established to reveal potential efficacy therapy for modulating immune response and inflammation (Suchner et al. 2000). Recently, a variety of dietary peptides and AAs derived from those foods are used to investigate their bioactivities, or therapeutic potential. An increasing number of novel dietary peptides have been identified as immune-nutrients which exert immune-regulatory or anti-inflammatory effects on the human gut system (Gill et al. 2000; Shimizu 2004 and Katayama et al. 2006). The involvement of AAs in metabolism is well known, where they support the essential bioactivities and functions of a living organism. For example, glutamine and glycine are important precursors for the biosynthesis of functional molecules, such as glutathione (GSH). In addition, the non-nutritional functionality of AAs has also attracted much attention from researchers. AAs are involved in signaling pathways and interact with various biological systems, including the endocrine, immune, and muscular systems as regulatory substrates (Suchner et al. 2000). Thus, it is critical to unravel the functionality and the impact of dietary peptides and AAs on a disease condition.

The goal of this review is to provide an overview of dietary peptides and AAs and their roles in anti-inflammation and antioxidant activities, as well as the potential application of dietary peptides and AAs to treat IBD. In addition, we discuss the underlying mechanisms that are beneficial for the functionality of dietary peptides and AAs.

Inflammatory bowel disease (IBD)

IBD is an inflammatory disease occurring in the small and large intestines. CD and UC are the two main forms of IBD (Cho 2006). The differences between these two diseases are based on the location and nature of inflammation. CD develops along the GI tract and affects all layers of the intestinal wall, while UC is restricted to the colon and affects only the mucosa. A defective gut epithelial barrier is usually associated with the development of IBD and leads to increasing the uptake of harmful adjuvants and antigens which result in an over-stimulated innate and acquired immune system. This triggers chronic inflammation and accelerating deterioration of the disease state (Sartor 2006).

Due to various environmental changes, the prevalence of IBD has increased in the past 3–4 decades (Cho 2006). Currently, about 1.4 million people suffer from IBD in the United States (CDC 2011). Previously, the prevalence rate of IBD was believed to be high in North America and Northern Europe. However, more recently the prevalence rate has begun to rise in low-incidence areas such as Southern Europe and Asia. The causes of IBD are still unclear; however, several factors were found to induce the development of IBD, including environmental, genetic, luminal microbial, and immunological factors. These factors interact with each other to establish an individual's susceptibility to IBD (Sartor 2006).

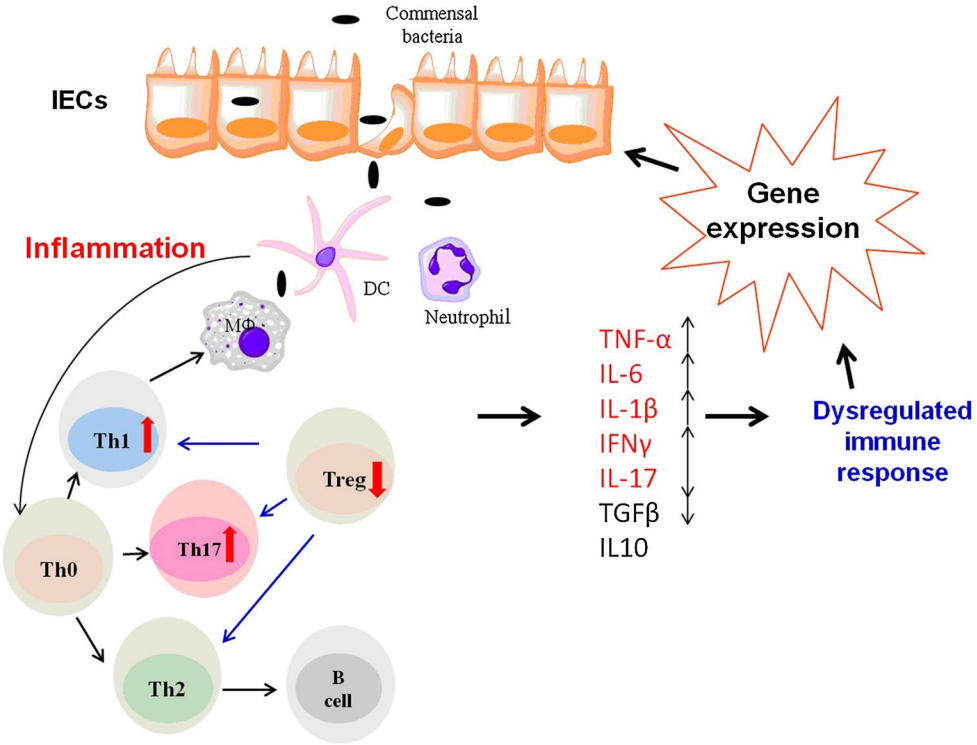

Fig. 1 Schematic representation of the pathogenesis involved with IBD

Pathogenesis of IBD

The pathogenesis of IBD is induced by complex genetic and environmental etiological influences, which leads to an increased immune responses and persistent inflammation in the intestinal epithelia and the gut-associated lymphoid tissues (GALT) (Fig. 1) (Othman et al. 2008). CD and UC have distinct characteristic immune pathogenesis. CD is more likely associated with the Th1 type of immune response and commonly affects the ileum and the colon. In contrast, UC may result from excessively stimulated Th2 responses and is essentially localized to the rectum. It is important to develop effective strategies for IBD disease prevention and treatment by thoroughly understanding the pathophysiological nature of this disease. As discussed by Sartor (2007), there are three main factors that account for the increase in IBD prevalence: commensal enteric bacteria, dietary factors, and genetic susceptibility. This has been demonstrated by the increase in the migrating population and alternative dietary composition that correlates with the prevalence of IBD diseases in the past five decades. The environmental influences are complex, and their impact on developing autoimmune diseases is not negligible. The persistent stimulus of enteric bacteria and dietary factors are capable of triggering the chronic inflammatory immune response in the genetically susceptible population. Genetic susceptibility is believed to be the intrinsic factor that leads to local pathogenic immune responses to commensal bacterial antigens.

The intestinal barrier consists of epithelial cells that maintain the physiological function, which is disrupted during the inflammatory process of IBD. The loss of barrier integrity allows bacterial translocation from luminal region into the lamina propria. Luminal bacteria that excessively permeated into the mucosa trigger the perpetuating inflammatory response by means of recruiting dendritic cells (DC), macrophages, and other immune cells to augment the release of inflammatory mediators and activate the innate immune response. During this process, secretion of pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, interferon (IFN)-γ and IL-17 are increased, while anti-inflammatory cytokines transforming growth factor (TGF)-β and IL-10 are reduced. As a result, increased Th1 and Th17 response and reduced Treg response develop. The impaired immune tolerance in IBD aggravates perpetuating inflammation which further leads to loss of integrity of the intestinal barrier (Fig. 1).

Dysregulated immune responses

The dysregulated immune system and chronic inflammation in the gut play a key role in the propagation of IBD. The following mechanisms explain the pathogenic immune response associated with IBD (Sartor 2006): (1) cytokines, such as TNF-α and IFN-**γ**, stimulate the release of pro-inflammatory mediators involved in developing and perpetuating inflammation in IBD; (2) the recruitment of macrophages is triggered by up-regulated expression of

recognition receptors and enhanced by pro-inflammatory cytokines, which are persistent in IBD and play a key role in amplifying severity of inflammation; (3) commensal bacterial antigens and adjuvants activate the NF-ts activated by pro-inflammatory cytokines, to amplify inflammation. This triggers the stimulation of naïve DC to initiate adaptive immune responses in the mucosal immune system; (4) overall, the failure of antigen elimination and persistence of various non-specific biological amplification mechanisms contribute to the development of inordinate humoral immune responses, resulting in an autoimmune disease. This situation is more significant in UC patients, as the non-specific antigen-induced autoantibody response is essential to stimulate the Th2 response in IBD that leads to impairing homeostasis of the intestinal immune system (Brandtzaeg et al. 2006). The loss of immune tolerance as a result of the depletion of Treg response is significantly associated with the pathogenesis of IBD as well as other autoimmune diseases.

Gut inflammation

In the GI tract, the luminal surface is the outside layer of the intestine where the digested food encounters the intestinal epithelium and nutrients are delivered into the body. The intestinal mucosa surrounds the intestinal lumen and comprises intestinal epithelial cells (IECs) The above mentioned alimentary layers are most susceptible to pathogenic infection due to the direct exposure to foreign antigens that exist in the external environment (Johnson 2001). After eliminating pathogens, the immune regulatory response is initiated to restore homeostasis of the intestinal mucosal immune system. The physiology and biological functions of the gut have strong implications for the maintenance of human health. However, the risks of malnutrition and susceptibility for the development of other systemic disorders are increased when gut health is deteriorated. Recruitment of neutrophils in the intestinal mucosa at the early stage of the immune response plays a critical role in initiating inflammatory processes. During the inflammatory response in the gut, the excessive secretion of pro-inflammatory cytokines can stimulate immune cells to produce more inflammatory mediators, including reactive oxygen intermediates that eventually result in intestinal tissue injury, the increase of mucosal permeability, granuloma formation, and amplification of persistent inflammation (Papadakis and Targan 2000). Consequently, chronic gut inflammation develops due to an over-activated Th1 immune response which leads to deterioration of the intestinal epithelium. Subsequently, substances such as enteric microflora and food debris permeate into the intestinal mucosal layer to trigger a complex of innate and adaptive immune responses. As a result, the homeostasis between the mucosal immune system and the commensal microflora in the gut is impaired. Meanwhile, the immune regulatory response that helps to prevent hypersensitivity to commensal microflora and innocuous food antigens is inhibited during the process of prolonged chronic inflammation. Due to the loss of immune tolerance, the Th1 response is continually amplified without suppression, resulting in the eventual dysregulation of the gut immune system.

In general, chronic inflammation in the gut results from the excessive pro-inflammatory cytokine production and persistent macrophage activation. Colitis develops in the intestine during the progression of chronic inflammation. It has been noted that chronic inflammation in IBD has been linked with the development of colorectal cancer (Terzic et al. 2010). An enzyme with carcinogenic property, cyclooxygenase-2 (COX-2) can be activated by pro-inflammatory cytokines such as IL-1-α/β and TNF-α in the intestine during the progression of chronic inflammation. COX-2 is recognized as precursors of tumorigenesis by which STAT3 is activated to stimulate tumor growth and activation of NF-κB to inhibit apoptosis (Macarthur et al. 2004 and Terzic et al. 2010). Meanwhile, production of ROS from inflammatory immune cells is able to cause DNA damage in epithelial cells, which results in genomic alterations and colon carcinogenesis (Meria et al. 2008). In IBD patients, there is a strong association between chronic inflammation and the development of colorectal cancer. Consequently, it is crucial to modulate chronic inflammation in the gut system, thereby preventing tumorigenesis.

Key cytokines mediating activation of inflammatory signaling events in IBD

The immune cells or tissues releasing cytokines aim to stimulate the immune system in response to micro-environmental changes. However, the loss of immune tolerance and increase in inflammatory cytokine production leads to perpetuated inflammation, which results in impairing the barrier integrity in the gut. A dramatically elevated secretion of IFN-**γ** aggravates chronic inflammation in the gut system (Fuss et al. 1996). The loss of immune tolerance and increase in inflamed macrophages and monocytes plays a crucial role in up-regulating Th1-dependent inflammatory response via producing TNF-α in the gut (Papadakis and Targan 2000). TNF-α is involved in the initiation and amplification of the inflammatory response in the intestinal epithelial system through directly triggering the production of other cytokines, chemokines, and endothelial adhesion molecules, as well as increasing vascular permeability, that leads to impairing the intestinal barrier integrity (Deventer 1997). A significant increase in TNF-α secretion in LP cells in CD patients has been observed, emphasizing its recruitment of neutrophils to

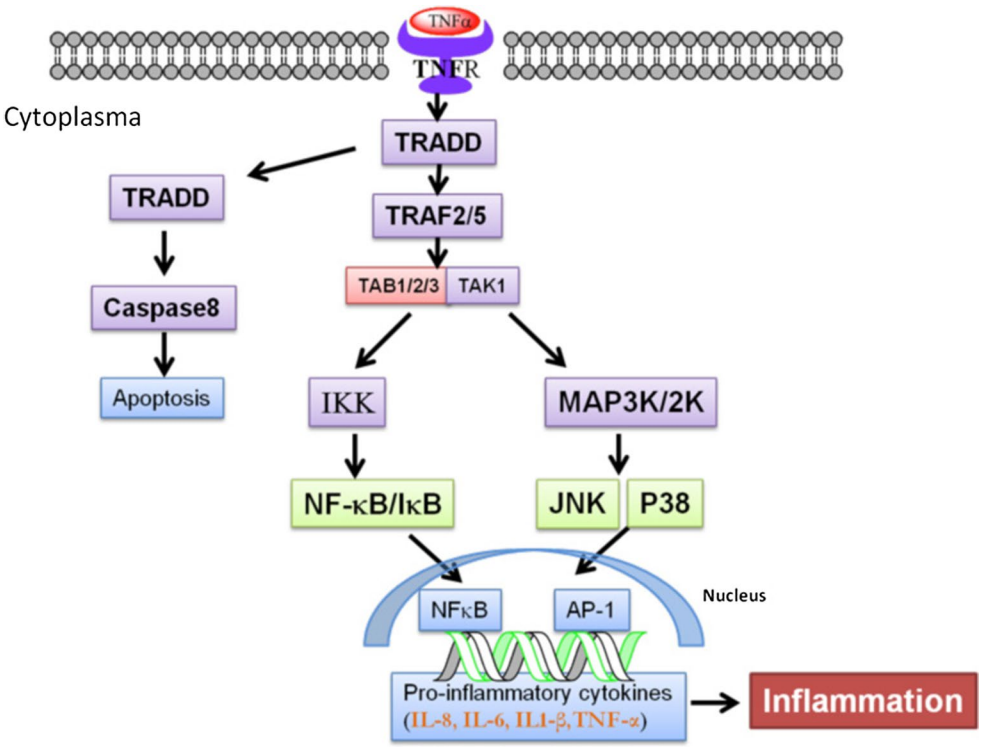

Fig. 2 Schematic representation of TNF-α signaling pathway

the site of inflammation and the enhancement of the Th1-dependent inflammatory reaction (Breese et al. 1994). A feature of increased TNF-α production mediating recruitment of neutrophils to the site of inflammation antigens suggests the predominant role of TNF-α and the enhancement of the Th1-dependent inflammatory reaction (Breese et al. 1994). Biological therapies, including infliximab, adalimumab, and certolizumab pegol, which are based on using monoclonal anti-TNF-α antibody to block TNF-α-mediated signaling events, have shown promise for IBD treatment by inhibiting perpetuation of chronic inflammation and inducing recovery of colitis in the gut (van Dullemen et al. 1995; Fuss et al. 1996; van Deventer 1999; Pache et al. 2009).

A basic mechanism underlying TNF-α induced inflammatory cascades stimulate activation of the cellular apoptosis/anti-inflammation and anti-apoptosis/inflammation, through signaling transduction following TNF-α binding with tumor-necrosis factor receptors (TNFRs) (Aggarwal 2003). TRAFs are recruited to bind with TNFRs to initiate the downstream signaling events following TNF-α stimulation (Jobin et al. 1999). Expression of TNFRs is widely distributed along the GI tract and is up-regulated during inflammation. TNFRs can trigger the intracellular signaling cascades via sensing exogenous TNF-α. TNF-α binding with TNFR1 induces activation of TRADD and subsequent recruitment of TRAF2/5 and FADD. FADD-activated caspase 8 leads to apoptosis. Meanwhile, the receptor-interacting protein (RIP) detaches from TRAFs and forms a complex with TAK1, TAB 1, and TAB 2 in cytoplasm after TRAF2/5 activation. This complex intermediates subsequent activation of NF-κB, JNK, p38 MAPK, and nuclear factor of activated T cells (NFAT) pathways (Fig. 2) (Ninomiya-Tsuji et al. 1999; Wang et al. 2001; Bouwmeester et al. 2004; Liu et al. 2009). Within the complex, TAK1 is an indispensable intermediate in TNF-α induced signaling pathways, and TNF-α stimulated TAK1 activation is involved in triggering AP-1 and NF-κB signaling transductions, thereby amplifying the inflammatory response (Baud and Karin 2001). TAK1 has been identified as a master regulator involved in maintaining homeostasis in immune cells, keratinocytes, intestinal epithelial cells, and hepatocytes (Omori et al. 2006; Kajino-Sakamoto et al. 2008; Inokuchi et al. 2010; Ajibade et al. 2012). Because the TNFR-mediated signaling cascade is mainly involved in governing pro-inflammatory signal transduction, the dysregulation of TNF-α–mediated signaling has a strong impact on impairing immune homeostasis. Neutralization of TNF-α contributes to maintaining the integrity of mucosal barrier and inhibiting chronic activation of the apoptotic pathway (Grell 1995). Furthermore, intestinal inflammation was shown to be attenuated by deletion of both TNFR1 and TAK1, which suggested that the TNFR signaling cascade was activated by TNF-α (Kim et al. 2009a, b) and indicated that the TNFR-mediated signaling pathway is involved in the pathogenesis of IBD.

Animal models for IBD

A variety of animal models have been developed to study IBD to mimic progression of disease so that the pathophysiology and etiology of human IBD can be studied in-depth and interventions for IBD therapy can be developed. In particular, animal models have been helpful in detecting efficacy of new pharmacological or immunomodulatory treatments, and more recently nutraceutical treatment. The animal models of IBD can be classified into five categories including chemically induced models, spontaneous models, cell-transfer models, gene mutation models, and genetically engineered models (Mizoguchi 2012). Genetically modified mice can spontaneously develop gut inflammation, which is not only caused by lacking genes but also by overexpressing a susceptible gene linked with IBD. A series of genetically engineered mouse strains have been generated that spontaneously develop IBD and are widely used in current research, such as mice lacking genes for IL-10 or tumor necrosis factor superfamily member (TNFSF)-15. The chemically induced model is more popular than other models of IBD. The main advantages of the chemically induced model are that it is affordable and easy to conduct without high technical requirements when compared to genetically engineered models. It is also the preferred method of inducing colitis because the severity of colitis can be controlled. Two exogenous chemical agents, including dextran sodium sulfate (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS), are widely used to induce gut inflammation in animals (Jurjus et al. 2004). These two exogenous chemical agents have been shown to cause alterations in immune response, gut physiology, and gut morphology in the experimental animals, inducing signs and symptoms characteristic of IBD. The mechanism by which these two agents induce colitis in the gut differs. TNBS produces symptoms resembling CD because of a dominating Th1 response resulting in mucosal inflammation (Te Velde et al. 2006). DSS is a polysaccharide and able to induce mucosal erosions and epithelial regeneration, which results in disease similar to human UC. DSS as an orally administered agent has been shown to cause inflammation specifically in the large intestine. However, the exact mechanism of how DSS induces colitis in animal models remains uncertain.

Murine models of IBD have been widely applied in research for over 50 years, and information regarding the fundamental mechanism of IBD has been obtained from such studies (Born and Bouma 2004). The most beneficial aspect of murine models of IBD is the possibility to study a target gene or protein in a genetically modified animal and for pre-clinical trials. This leads to specifically focusing on studying the function of a susceptible gene in the disease progression of IBD or the mechanism underlying a novel therapeutic agent for IBD treatment. The fundamental knowledge about genetic factors of IBD and the mucosal immune system has been obtained from current studies using murine models of IBD. Although murine models provide an in vivo model similar to the human system in complexity, the structure and function of the murine digestive system are different from those of humans. Accordingly, there is a demand for establishing a model that is more representative of the human gut system and mucosal immune responses during chronic inflammation.

The physiological composition and functions of organs in pigs are more similar to those of humans when compared with rodents. Pigs particularly have a remarkably comparable digestive tract, nutritional requirements and metabolism, and bone development when compared with humans (Casteel et al. 1996). Furthermore, the intestine development of newborn pigs is similar to that of humans and significantly different from rodents (Reeds and Odle 1996). As such, immature pigs have been used successfully as a model for the gastrointestinal function of children. The piglet has an equivalent growth rate with humans, so they can be used to investigate long-term nutrition impact research. Pigs have analogous mucosal immune systems with humans, since porcine mucosal immune systems have well organized and compatible GALT which includes epithelial cells, Peyer's patches, and LP. Moreover, the intestinal mucosa of pigs contains dendritic cells (DC) which are functional as antigen-presenting cells (Charerntantanakul and Roth 2007). Porcine intestinal DC has a compatible function with human follicle-associated epithelium M cells which are able to process antigen and present it to T cells. Pigs also have equivalent cellular immune responses with humans. Charerntantanakul and Roth (2007) demonstrated that porcine T cells can recognize antigens in a major histocompatibility complex (MHC). Antigen-presenting cells expressing MHC II are largely found in the pig jejuna LP and endothelial cells (Bailey and Haverson 2006). Moreover, Fossum (1998) and Sipos et al. (2004) indicated that pigs can also produce transcription factors and pro-inflammatory cytokines such as IL-8 and TNF-α. Taken together, the porcine model of IBD is particularly beneficial for studying the complexity of mucosal immune responses during gut inflammation due to similarities to the human immune system and mechanisms for digestion. The porcine model has been applied for preclinical study of IBD therapies which contributes to further design of human clinical trials.

Therapeutic approaches in IBD

The complicated interplay of genetic, environmental, immunological, and lifestyle factors leads to the induction of excessive immune responses and persistent inflammation in the intestinal epithelia and gut-associated lymphoid

tissues, which further results in development of IBD. Except for immune-suppressing therapies, the current treatments for IBD are based on modulation of inflammation and immune responses. Drug-based therapies mainly contribute to maintaining IBD patients in the remission phase. Meanwhile, surgeries are more effective for UC than CD treatment, by which the deteriorated parts of the colon or rectum are removed. To date, there is no effective medical therapy to cure IBD. Surgical management is used to control severe disease progression which leads to developing extensive intestinal damage. Due to unclear etiology and autoimmune disorder characteristics, removing of the lesion section of the intestine or colon does not prevent relapses of IBD.

Due to the limitation of conventional medical treatments, researchers have focused on exploring novel therapeutic strategies for IBD treatment. Dietary components generally have anti-inflammatory and anti-oxidative properties to potentially ameliorate the disease conditions of IBD. Current dietary interventions for IBD treatment mainly include probiotics, prebiotics, AAs, and dietary peptides. Dietary interventions may not be suitable for replacing conventional medical therapy, but are effective complementary approaches for IBD treatment.

Comparing conventional treatments and dietary intervention for IBD treatment

The step-up and top-down approaches illustrate the algorithm of IBD treatments, which consists of immunomodulators, anti-inflammatory medicines, and biological reagents (Papa et al. 2009). The step-up approach is a conventional strategy used for IBD treatment in which the patients are initially treated with a moderate drug, then forwarded to stronger ones to control disease progression (Papa et al. 2009). This approach is initiated with an anti-inflammatory drug such as 5-aminosalicylates and antibiotics. The immunosuppressive drugs such as corticosteroids are administered to IBD patients in the second phase of treatment. In the third phase, immunomodulatory drugs including azathioprine and 6-mercaptopurine are administered to patients to maintain remission. Finally, Infliximab, a monoclonal anti-TNF-α antibody, is used to block TNF-α-induced pro-inflammatory response and disease progression. Infliximab is generally used for severe cases of IBD when the patients fail to respond to the aforementioned pharmacological therapies. On the other hand, the steps of treatment progression in the top-down approach are reversed from the step-up approach. Current studies have demonstrated that the prolonged improvement and healing of the mucosal system in CD patients are results of the top-down strategy (Hommes et al. 2006). Since all of the above mentioned treatments are mainly based on immunosuppression, strong side-effects are associated with the long-term use of immunosuppressive agents, which leads to ineffective maintenance of the remission phase and an increase in the relapse rate (Owenberg et al. 2006). In addition, the conventional medical therapies for IBD have significant side-effects including increased risk of lymphoma, infectious complications, endocrine impairment, and hypertension (Baumgart and Sandborn 2007). Meanwhile, effective and safe dosage levels for use of the above drugs to treat IBD are still controversial and may lead to an increase in toxicity risk factor.

Because dietary compounds have a direct impact on gut health as a source of luminal antigens, various dietary interventions or supplements have been found with beneficial effects in maintaining remission or improving recovery of lesions in IBD patients. The efficacy of dietary interventions is more promising to maintain clinical remission when systemically combined with medical treatments for IBD. Other important therapeutic remedy besides the medical environment is improved nutrition. Due to the deteriorated mucosal barrier and progressive lesion development, nutritional status in patients suffering from IBD is significantly compromised. Accordingly, dietary interventions or nutritional supplementation can potentially contribute to modulating malnutrition status in IBD patients (Yamamoto 2013).

A variety of functional foods have been found with therapeutic potential or health-promoting properties beyond their nutritional function. The anti-inflammatory and anti-oxidative properties of functional foods, such as glutathione (GSH) (Oz et al. 2005; Kovacs-Nolan et al. 2014), polyunsaturated fatty acids (Bassaganya-Riera and Hontecillas 2006 and Chapkin et al. 2007), and AAs (Faure et al. 2006 and Ockenga 2005), were shown to have numerous beneficial effects in pre-clinical colitis models by modulating the immune system and reducing oxidative stress in human and animal subjects. For example, AA-based diets were shown to improve the intestinal permeability in patients suffering from CD (Teahon et al. 1991). Their further study also demonstrated the effectiveness of peptide-based diets in treating CD patients (Royall et al. 1994). Since dietary intervention for IBD treatment is less invasive compared to conventional medical approaches, prophylactic and non-pharmaceutical treatment of IBD is desirable.

Dietary peptides and AAs reduce oxidative stress in gut

As mentioned above AAs and peptides have also been shown to possess a number of biological activities in addition to their nutritional properties. Bioactive AAs and peptides are generated from food proteins through

gastrointestinal digestion, chemical and enzymatic hydrolysis, or bacterial metabolism. Numerous potential mechanisms were identified underlying antioxidant actions including (1) direct radical scavenging, (2) downregulation of radical production, (3) elimination of radical precursors, (4) metal chelation, (5) inhibition of xanthine oxidase, and 6) elevation of endogenous antioxidants (Disilvestro 2001). Supplementation with anti-oxidant AAs and peptides has been found to reduce oxidative stress in vitro and in vivo in animal disease models or cell models.

Several mechanisms exist in the intestine to cope with the electrophilic and oxidative stresses exerted by these xenobiotics while maintaining cellular integrity and tissue redox homeostasis. However, this redox homeostasis can be disrupted under chronic inflammation. The condition of increasing oxidative stress with decreasing antioxidant defenses has been identified in colonic mucosal biopsies of patients with IBD (Lih-Bordy et al. 1996). This condition of oxidative stress is generated from oxidative respiratory bursts during the phase of chronic intestinal inflammation. The oxidative respiratory burst is a characteristic mechanism of the immune response to eliminate bacteria which is induced by immune cells, including phagocytes, neutrophils, and macrophages. The endogenous antioxidative system, composed of numerous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR), protects the gut from free radical-induced oxidative damage. However, these endogenous antioxidants are overwhelmed by the persisting endogenous oxidative stress generated during chronic intestinal inflammation. Thus, the exogenous antioxidants generated from foods are optimal candidates for enhancing the function of the antioxidant defense system in the gut system when experiencing chronic inflammation.

Egg yolk and soy peptides reduce oxidative stress in the gut

The potential candidates for antioxidant peptides are determined by the content of AAs with strong capability for ROS scavenging. Although the antioxidant capacity of peptides depends on the functional side chain, the molecular conformation and intramolecular bonds have significant impact on the degree of oxidation. It has been recently found that the physicochemical properties of the C-terminal and N-terminal regions have an impact on the antioxidant potency of the peptide. The antioxidant efficacy of the functional peptides is determined by AAs at C-terminal regions (Li and Li 2013). Antioxidant peptides play an important role to protect cellular membrane from lipid oxidative damage when they are more oxidatively labile than unsaturated fatty acids and closer to the site of ROS generation.

His-rich soy peptides were recently identified to exert antioxidant activity by chelating metals, scavenging free radicals, and quenching active oxygen molecules (Chen et al. 1995, 1998). The oxidant quenching ability of the peptides can be increased by the presence of antioxidant AAs such as histidine (His), tyrosine (Tyr), tryptophan (Trp), methionine (Met), and lysine (Lys) (Saito et al. 2003). The oligophosphopeptides derived from hen egg yolk were shown to inhibit IL-8 secretion in H_2O_2-treated Caco-2 cells while increasing GSH levels and elevating glutathione S-transferase (GST), catalase (CAT), and o-glutamylcysteine synthetase (γ-GCS) activities (Katayama et al. 2006, 2007). Hydrolysates from milk proteins, including whey protein and casein, have been identified with antioxidative properties, by preventing liposome oxidation and scavenging superoxide anions and free radicals (Pena-Ramos and Xiong 2001 and Suetsuna et al. 2000).

AAs reduce oxidative stress in the gut

The functionalities of AAs play an important role in various aspects of health and disease. The vulnerability of AAs to ROS oxidation primarily depends on their side chain property. All AAs are theoretically oxidizable, but the most oxidizable include those with nucleophilic sulfur-containing side chains such as cysteine (Cys) and methionin (Met) or aromatic side chains like Trp, Tyr, and phenylalanine (Phe) (Elias et al. 2008). The exogenous antioxidative activities of AAs are determined by their overall ROS scavenging activity.

Accordingly, the sulfur-containing AAs (SAA), including Cys, Met, and taurine (Tau), a Cys metabolic product, have strong antioxidant activities due to their essential thiol group. The L-Cys reacts with L-glutamic acid and glycine to synthesize GSH. Because it contains a thiol group, GSH is a vital endogenous antioxidant. GSH metabolism also contributes to restoring the redox homeostasis in the body and protecting tissues from oxidative damage caused by ROS produced during immune responses. Tau is the end product of Cys metabolism produced mainly in liver, which is the most abundant amino containing sulfonic acid. Tau in itself has anti-oxidant properties and has also been found to have immunomodulatory activities. Ronchi et al. (2010) found that Met supplemented to mice fed a protein-free diet reduced ROS levels and increased GSH concentrations thereby restoring redox balance in liver. Furthermore, certain AAs have been shown to exhibit antioxidative stress properties in the gut system. H_2O_2-stimulated oxidative stress in Caco-2 intestinal epithelial cells was shown to be inhibited after pre-treatment with Cys, valine (Val), isoleucine (Ile), leucine (Leu), Trp, His, lysine (Lys) or alanine (Ala) (Son et al. 2005; Katayama and Mine 2007). Tau supplementation in a TNBS-induced mouse colitis model

was shown to reduce myeloperoxidase (MPO) activity and decrease basal and formyl-methioyl leucyl phenylalanine (FMLP)-induced reactive oxygen generation in colon tissue (Son et al. 1998). The results suggested that administration of Tau restored oxidative defense activity in the colon, thereby reducing gut inflammation.

Anti-inflammatory properties of dietary peptides and AAs

Dietary peptides modulating gut inflammatory responses

Dietary peptides derived from food proteins have bioactive properties that are beneficial to disease prevention and health enhancement. The peptides derived from food proteins may vary in molecular size, net charge, and solubility depending on the AAs they contain; and peptides could easily pass through the intestinal lumen into the mucosa to interact with target biological molecules such as membrane receptors. In addition, dietary peptides derived from food proteins with therapeutic capacity can also be delivered into the colonic epithelial cells to attenuate inflammation and modulate pro-inflammatory signaling events. A soy-derived tri-peptide was recently identified with anti-inflammatory activity to modulate severity of colitis and suppress secretion of pro-inflammatory cytokines via an intestinal proton-dependent peptide transporter (h-PepT1) (Kovacs-Nolan et al. 2012 and Young et al. 2012). Also, dietary peptides derived from whey protein have been identified with anti-inflammatory properties to inhibit IL-8 production from lipopolysaccharide (LPS)-induced respiratory epithelial cells by blocking LPS binding with TLR4 (Iskandar et al. 2013). Administration of a dietary Ala-Gln dipeptide was shown to reduce expression of inflammatory mediators and elevate expression of mucin 2 and heat shock protein 72, thereby enhancing the recovery of mucosa in a DSS-induced mouse model (Hou et al. 2013). Likewise, other therapeutic targets deserve attention from researchers to identify effective mechanisms by which dietary peptides can regulate the cellular signaling events relevant to inflammatory responses produced by IECs. γ-glutamyl peptides, including γ-glutamyl cysteine (γ-EC) and γ-glutamyl valine (γ-EV), have been developed as a flavor enhancer by Ajinomoto Co. Ltd. and demonstrated capacity to induce calcium-sensing receptor (CaSR) activation and elevation of intracellular calcium levels (Conigrave and Brown 2006; Wang et al. 2006a, b; Ohsu et al. 2010). γ-EC and γ-EV are both potential allosteric agonists that regulate the integrated CaSR-mediated cellular signaling events. More recently, we have shown that γ-EC and γ-EV have an effect on inhibiting inflammation in both in vitro and in vivo IBD models (Zhang et al., unpublished).

Anti-inflammatory and anti-apoptotic effects of AAs in the gut system

AAs have long been known for their essential role in biological life because they are the building blocks of all protein structures. The non-nutritional functionality of AAs has been the focus of many nutraceutical and functional food studies. AA metabolism and synthesis are closely linked to various body systems, including the endocrine system, immune system, and muscular system. However, AA metabolism is significantly altered during disease states. The immune-modulatory effects and cell signaling functions of AAs have been extensively investigated and play a critical role in various aspects of health and disease. Trp has been the focus in recent years because of its involvement in immune suppression. Trp catabolism through the kenurenine metabolic pathway is involved in modulating homeostasis of the immune system, due to the activated tryptophan catabolic enzyme indoleamine 2,3-dioxygenase (IDO) (Moffett and Namboodiri 2003). The toxic metabolites from kynurenine metabolic pathway can induce apoptosis of Th1 cells, thereby inhibiting Th1-mediated inflammation. The downstream metabolites of IDO may stimulate the native T-cells to develop into Treg cells and increase the production of immunosuppressive cytokines. Over-expression of IDO, stimulated by pro-inflammatory cytokines and a decrease of Trp, has been found in human IBD patients (Wolf et al. 2004). The therapeutic function of Trp was demonstrated in a DSS-induced porcine IBD model (Kim et al. 2010). As shown in this study, a decrease of pro-inflammatory cytokine expression indicated that the Th1-mediated inflammation of IBD was inhibited by treatment with Trp. This result may be caused by Trp metabolism inducing apoptosis in T cells. Trp has also been shown to have CaSR agonistic activity (Wang et al. 2011), and we recently demonstrated that Trp supplementation inhibited IL-8 production from inflamed intestinal epithelial cells in a CaSR-dependent manner (Zhang et al., unpublished). Interestingly, our recent data showed that Trp and the dipeptide γ-EC can block a well-defined mitochondria-mediated apoptosis, which was initiated by the overexpression of *PUMA* or *APOL6 (apolipoprotein L6)*, two of the BH3-only pro-death genes, in human intestinal epithelial cells. We further showed that both Trp and γ-EC can initiate autophagic survival mechanism that counters apoptosis (Hu et al., unpublished observation). Importantly, it is well documented that AA metabolism and signaling are intimately related to (macro)autophagy. Autophagy (literally hed observation). Importantly, it is highly regulated, intracellular lysosome-dependent homeostatic pathway to eliminate and recycle damaged/unwanted proteins and organelles. Autophagy is also used to remove intracellular pathogens, to facilitate innate immunity, and to modulate cell death.

Several signaling pathways, for example, p53 and NF-κB, can be activated as AA/nutrient deprivation to microbial invasion, and converge to regulate autophagy at multiple stages of the process. In essence, several functional interactomes/complexes, for example, ULK1- Atg13-FIP200, Beclin1-Vps34-Vps15, Atg12-Atg5-Atg16L, are required for the biosynthesis of two adaptor molecules, phosphatidyl-inositol 3-phosphate (PI3P), and microtubule-associated protein light chain 3II phosphatidyl ethanolamine conjugates LC3II-PE, which are necessary for the progression and completion of autophagic vesicle (autophagosome, amphisome, and autiolysosome) formation (Itakura et al. 2012). Interestingly, glutamine (Gln) has been recognized as a promising therapeutic candidate for improvement of gut health and has been intensively studied for IBD treatment based on its effects on repairing the epithelial layers, maintaining intestinal mucosa function, and enhancing the immune responsiveness (Kretzmann et al. 2008). Beutheu et al. (2013) determined that both glutamine and arginine supplementation had protective effects on maintenance of intestinal barrier integrity in a methotrexate (MTX)-induced Caco-2 cell model via regulation of activation of JNK, extracellular signal-regulated kinases (ERK1/2), and NF-κB. Gln supplementation has also been found to inhibit inflammatory responses in the experimental colitis model by regulating NF-κB activation (Fillmann et al. 2007). Sakiyama and his colleague (2009) identified that intestinal epithelial cells treated with Gln under conditions of heat stress led to an increase in the expression of LC3-II which is correlated with autophagy activity and autophagosome formation. In addition, they also found that Gln supplementation in intestinal epithelial cells experiencing heat stress resulted in an inactivation of mTOR/p38 MAPK pathway, which has the major inhibitory effect on autophagy.

SAAs play a critical role in regulating the immune responses of mammalian body systems. An increase in dietary Cys resulted in the enhancement of mucin synthesis in a DSS-treated rat model (Faure et al. 2006). Mucin, Cys-rich glycoproteins secreted by goblet cells, is constituted into a viscoelastic film to support the intestinal epithelial integrity to protect the intestinal epithelium from damage caused by digestive fluid, microorganisms, and toxins. Kim et al. (2009a) also found that supplementation of L-Cys in a porcine model of DSS-induced colitis resulted in a reduction of local chemokine and pro-inflammatory cytokine expression and neutrophil influx, thereby attenuating the intestinal inflammatory responses and restoring immune homeostasis. Tau in itself has anti-oxidant properties and has also been shown to possess immunomodulatory activities. TauCl plays a vital role in regulating pro-inflammatory cytokine production which implicates an involvement of signaling mechanisms. A possible mechanism for regulation of signal transduction by TauCl is its ability to induce oxidation of I-κB-α, thereby directly inhibiting the activation of NF- κB (Kanayama et al. 2002).

Novel cellular mechanisms underlying inhibition of inflammatory responses in the gut by dietary peptides and AAs

Dietary AA- and peptide-based interventions have been shown to have therapeutic efficacy to attenuate intestinal inflammation (Kovacs-Nolan et al. 2012). Those interventions have a limited toxic effect compared with the pharmaceutical approaches used to treat IBD patients. The AAs or peptides are potentially involved in regulating cellular signal transduction and gene expression by interacting with cellular membrane receptors or being transported into cells. However, their mechanisms may be varied and depend on their functionalities or conformational structures. Therefore, it is still necessary for researchers to explore new biological mechanisms by which AAs or peptides modulate inflammatory signaling events to reduce inflammation.

Reduction of inflammation by dietary peptides mediated by proton-dependent peptide transporter (hPepT)-1

Currently, dietary peptides have been identified with immunoregulatory activities (Gill et al. 2000). However, most studies are based on analyzing the bioactive effects of dietary peptides on immune cells and the resulting cytokine production. The relevance of intracellular signal transduction influenced by dietary peptides has not been adequately examined, perhaps because conventional understanding of peptide absorption and metabolism is limited. The conventional belief is that after food proteins are ingested, they are hydrolyzed into oligopeptides by a variety of proteinases in the GI tract and then further hydrolyzed into AAs by peptidases at the brush-border (Shimizu 2004). This theory has been challenged recently by identification of a novel peptide-specific transporter along the intestinal epithelial cell membrane called the intestinal proton-dependent peptide transporter (hPepT1) (Adibi 1997; Shimizu 2004). The hPepT1 belongs to the solute carrier (SLC)-15 family, an electrogenic transporter located on the cellular membrane that produces a proton gradient to accelerate oligopeptide transport into cells (Daniel and Kottra 2004). It provides an energy-saving route for the uptake of AAs in peptide form by cells. The function of hPepT1 is to transport di- or tripeptides across the bush border and into enterocytes, where they are converted into either AAs or released; they may also pass through IECs and enter the portal circulation via. hPepT1 delivery (Adibi 1997). The transporting ability of hPepT1 in the intestine is reduced by any altered substrate

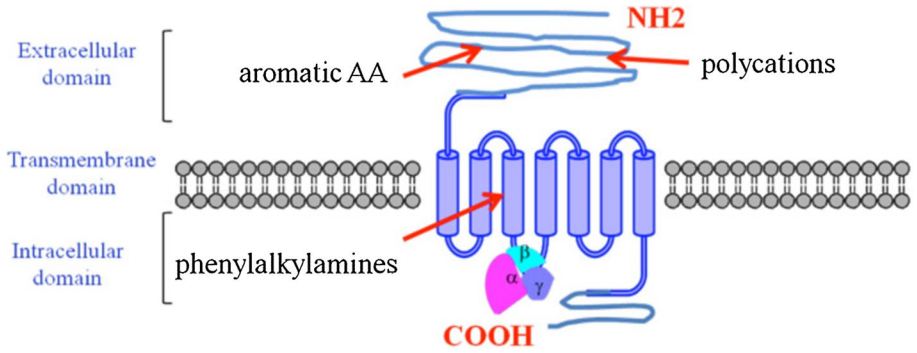

Fig. 3 A schematic representation of the monomer of CaSR

affinity during a chronic inflammatory condition, which is possibly caused by the destruction of the intestinal barrier integrity (Sundaram et al. 2007). The relevance of hPepT1 transporting short peptides into IECs is the possible mechanism underlying dietary peptide-regulating activities in IECs.

Previous studies showed that hPepT1 potentially contributes to delivering anti-inflammatory oligopeptides into colonic cells to modulate the inflammatory responses. Son et al. (2008) identified the inhibitory effects of the peptide carnosine (β-Ala-His), which is derived from meat products, on modulating inflammatory responses in Caco-2 cells mediated by hPepT1 transportation. Kovacs-Nolan et al. (2012) demonstrated that the soy protein-derived tripeptide VPY can be transported into Caco-2 cells by h-Pept1 and has anti-inflammatory activity to decrease pro-inflammatory mediator expression in vitro and in vivo in mice. Therefore, hPepT1 might be an alternative mechanism by which short-chain dietary peptides exert their anti-inflammatory activity to attenuate the inflammatory responses in colonic epithelial cells.

Calcium-sensing receptor (CaSR)-mediated anti-inflammatory activity

The CaSR, referred to as a G protein-coupled receptor (GPCR), is composed of seven transmembrane-spanning domains and coupled with a G protein through intracellular loops and is able to sense changes in extracellular calcium concentration and subsequently modulate related cellular activities as shown in (Fig. 3) (Brown and MacLeod 2001). Expression of CaSR was first identified in the parathyroid gland with its characteristic function of maintenance of calcium homeostasis and regulation of parathyroid hormone (PTH) expression. Current studies have shown that CaSR is widely distributed in diverse types of cells within different tissues including the nervous system, bone, GI, and kidney. CaSR is involved in the regulation of various cellular activities including secretion, apoptosis, proliferation, differentiation, and ion-channel activity (Brown and MacLeod 2001; Mundy 2002; Hofer and Brown 2003). The extracellular domain consists of venus fly trap (VFT) which acts as the major ligand for agonistic interaction. The transmembrane domain is composed of the characteristic seven transmembrane helices. The carboxyl terminus is extended into the cytoplasm. The location of binding sites for agonists and antagonists is shown in Fig. 3.

Expression of CaSR has been identified in normal colonic epithelium and Caco-2 cells and enables colonic epithelial cells to sense extracellular Ca^{2+} concentrations (Chakrabarty et al. 2003). The expression of CaSR in colon from healthy gut has been studied. CaSR in the colonic epithelium can be activated by diet-derived CaSR agonists present in the colon such as polyamines (Quinn et al. 1997). This suggests that CaSR expressed in the colon may have a potent biological function to regulate gut health in response to available agonistic nutrients. The Ca^{2+}-induced activation of CaSR in colonic myofibroblasts had been shown to promote differentiation of these cells, thereby stimulating regeneration of the intestinal barrier (Pacheco and MacLeod 2008). A reduction of CaSR expression was identified in colon tissue in colorectal cancer patients (Chakrabarty et al. 2003). Increased dietary calcium intake leads to prevention of development of colorectal cancer and promotes colonic mucosal epithelial cell differentiation in CaSR-dependent fashion (Bresalier 1999; Hebert et al. 2004). Therefore, CaSR activation is known to have beneficial effects on maintaining colon integrity and reducing risk of development of colon cancer. This may also be involved in regulating the physiological changes in various aspects of the gut system to maintain homeostasis.

The GRK-β-arrestin coupled system has been identified as a critical mechanism to regulate GCPR activity in addition to the G-protein-mediated classical signaling regulatory loop. β-Arrestin-dependent desensitization is only initiated when the agonistic modulators bind with GCPR to induce GRK phosphorylation, thereby mediating interaction between bearrestins and the receptor (Pierce and Lefkowitz 2001). Furthermore, the role of β-arrestin as multifunctional scaffold and adaptor proteins has been explored, and they are involved in regulating numerous signaling networks including JNK, p38 MAPK, and ERK,

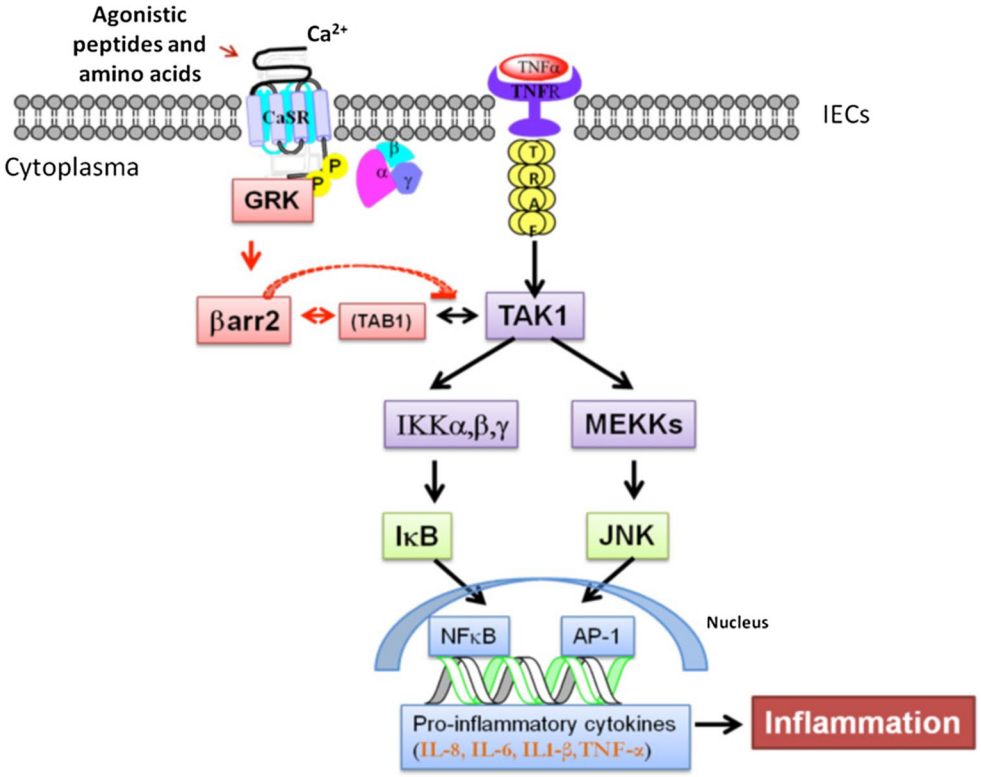

Fig. 4 Schematic diagram summarizing the mechanism of CaSR-mediated anti-inflammatory activity of CaSR agonistic peptides and amino acids and the TNF-α-induced inflammatory pathway

a serine-threonine protein kinase (Akt), PI3 K and Ras homolog family member A (RhoA) pathways (DeWire et al. 2007). As signaling scaffold proteins, β-arrestins have been discovered with capability of directly binding with key target molecules such as ERK1/2 and JNK, IκBα, as well as TRAF6 (Gao et al. 2004; Wang et al. 2006a, b). In addition, β-arrestins are able to regulate the immune response by coordinating NF-th key target signaling pathways in cells. β-arrestins are able to directly associate with I-κBα thereby inhibiting NF-κB-dependent signaling pathways in various cell types, such as HEK293, HEKTLR4, HeLa, THP-1 cells (Gao et al. 2004; Witherow et al. 2004; Fan et al. 2007). Accordingly, inflammation can be attenuated by recruitment of β-arrestin as a result of allosterically activated CaSR-induced GRK activation. Taken together, CaSR has a biological property to sense availability of nutrient agonists or allosteric modifiers by which bioactivities of the gastrointestinal epithelial cells can be regulated. Therefore, CaSR expressed along the GI tract may be a novel therapeutic target for CaSR agonistic peptides or AAs exerting beneficial effects on gut health.

Activation of CaSR in the intestinal epithelial system potentially contributes to enhancement of gut health. γ-glutamyl peptides or aromatic AAs have been identified with CaSR agonistic activity to allosterically activate CaSR located in the cellular membrane (Conigrave and Brown 2006; Wang et al. 2006a, b; Ohsu et al. 2010). In our recent studies, dietary γ-glutamyl dipeptides and AAs such as γ-EC, γ-EV, and Trp exerted potent anti-inflammatory effects in IECs to attenuate TNF-α-induced signaling events via allosteric ligand activation of CaSR, as shown in (Fig. 4). The underlying mechanism revealed by our study is that the activation of CaSR by the γ-glutamyl dipeptides leads to the association of β-arrestin-2 with TAB 1 to block activation of TNF-α-mediated pro-inflammatory signaling cascade through TNFR, a previously unknown function of CaSR. Since involvement of β-arrestin-2 was found to inhibit the TNF-α-dependent pro-inflammatory singaling cascade via cross-talk with the TNFR, activation of CaSR has potential benefits for ameliorating an impaired gut system (unpublished data). Therefore, CaSR expressed along the GI tract may be a novel therapeutic target for dietary peptides or amino acids exerting enhancive effects on gut health.

Conclusion

In summary, patients suffering from IBD experience chronic inflammation and associated augmentation of oxidative stress. It is well known that inflammation and oxidative stress play a key role in the development and propagation of IBD. Food components are now being appreciated for their chemoprotective properties and limited side effects when compared with conventional medical treatments. The chemoprotective and anti-apoptotic activity of dietary AAs and peptides and their role in attenuating intestinal

inflammation and restoring intestinal mucosal homeostasis have been demonstrated by recent studies in IBD models. The discovery of novel dietary-based interventions as well as the understanding of their mechanism of action offers new ways in which to avert the development of diseases.

Conflict of interest There is no conflict of interest.

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