Current evidence on the modulatory effects of food proteins and peptides in inflammation and gut microbiota

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ABSTRACT

Inflammation, oxidative stress, and immune dysbalance are common disturbed states at mucosal surfaces that underlay the pathology of several gastrointestinal disorders such as inflammatory bowel disease (IBD). Nowadays, gut health is not only regarded as the absence of gastrointestinal complaints but also a key approach to maintain general well-being status and prevent chronic illness. Foods and their bioactive compounds including proteins and bioactive peptides can influence the gastrointestinal health. This chapter provides some introductory concepts on gut homeostasis and the altered mechanisms on IBD, with the aim to describe the potential health benefits of food proteins and peptides at the gastrointestinal tract. The evidence achieved on the antioxidant, anti-inflammatory, and immunomodulatory activities by *in vitro* models is described. Despite the *in vivo* evidence is lower, those preventive effects demonstrated against intestinal inflammation in animal models and human trials are highlighted. Noteworthy, the involvement of the gut microbiota modulation as promising mechanism for food peptides to operate against intestinal inflammation is also summarized. This chapter encourages further research to decipher the relationship between food peptides and gut microbiota on intestinal inflammation, and ultimately advance into the development of food peptide-based functional compounds for IBD and other intestinal inflammatory disorders.

KEYWORDS: bioactive peptides, food proteins, gut microbiota, inflammatory bowel disease, intestinal mucosa, obesity.

1. Introduction: inflammation and oxidative stress

Inflammation is a step-by-step immune response of vascularized living tissue whose function is to enclose injury, destroy invading microorganisms, inactivate toxins, and to restore the tissue or organ for recovery (Calder et al., 2013). The inflammatory reactions triggered by inflammatory stimuli lead to the release of cell derived mediators such as interleukins (IL), tumor necrosis factor alpha (TNF-α), prostaglandins (PG), nitric oxide (NO), and leukotrienes (Dadar et al., 2019). These molecules mediate the inflammatory response by interacting with various cellular and subcellular components in different cell types. Properly controlled, regulated inflammatory responses are essential to keep body healthy, and maintain or restore homeostasis in damaged biological compartments. However, when inflammation becomes excessive, irreparable damage to host tissues and disease can occur. Thus, experimental, clinical and epidemiological studies have revealed that chronic inflammation contributes to the development of approximately 15-20% of malignancies worldwide, included inflammatory bowel disease (IBD), type-2 diabetes, obesity, arthritis, and cardiovascular diseases (Serhan & Savill, 2006; Wang et al., 2011). Together with inflammatory process, oxidative stress has been recognized as one of the most critical factors implicated in chronic diseases (Chung et al., 2009). During aerobic metabolism, oxygen may be partially reduced to yield reactive molecules termed reactive oxygen species (ROS). ROS participate in the bactericidal action of phagocytic cells and in the regulation of the immune environment in the body. ROS are also responsible for attacking cell nucleophilic centers causing lipid peroxidation, protein oxidation, and genetic alterations, including DNA damage, mutations, epigenetic changes, and genomic instability (Kan et al., 2008). Fortunately, the human body is equipped with an effective defense system to neutralize the toxicity of ROS. However, oxidative stress results from an imbalance provoked by either an over-

production of ROS, as in the case or persistent inflammation, or a defect in the cellular defense system.

2. Impact of "gut health" on "general human health"

The gastrointestinal (GI) tract represents a complex interface system between the body and the external environment, being permanently exposed to exogenous and endogenous factors. It has an essential role at absorbing water and nutrients for the support of bodily functions, thus contributing to ionic homeostasis.

Structurally, the gut consists of a stratified monolayer of differentiated epithelial cells. These cells are derived from stem cells within intestinal crypts which quickly replicate, differentiate, and migrate towards villi, replenishing the active turnover of epithelium. Epithelial cells can be divided into four main subsets including absorptive enterocytes, mucus-producing goblet cells, antimicrobial peptides-producing Paneth cells, and hormone-secreting enteroendocrine cells, being all of them connected via tight junction interactions which provide a physical border to the semi-permeable intestinal barrier. This continuous layer of cells constitutes an important physical and biochemical site of defense against food compounds, bacteria, toxins, and allergens. Along with this, immune cells present in lamina propria, which is the space beneath the epithelial cell monolayer, also recognize pathogens and allergens. To respond to external stimulants, epithelial and immune cells (mainly dendritic cells and macrophages) are cooperatively activated, thereby producing cytokines, chemokines, antimicrobial peptides, mucins, and other modulatory compounds that reinforce and restore the intestinal barrier (Wan et al., 2018).

The GI tract also keeps at bay a rather large number of microorganisms (and microbial molecules) present at the lumen, which form the microbiota. This gut microbiome is known to interact with the host in a symbiotic way, exerting a variety of

beneficial effects including digestion of substrates and nutrients production, development, maturation and regulation of the immune system, and prevention of the growth of harmful microorganisms (Aleksandrova et al., 2017).

Maintenance of the intestinal barrier function appears to be of paramount importance for the host. Recent advances have discovered that gut has much far-reaching impact, more than food processing and nutrient uptake, on the overall good health of the body: immune tolerance, defense against infections, and signaling to the brain. With accumulating evidence from animal experiments and epidemiological studies, "gut health" is now regarded as a new objective, not only a target of treatment to widespread gastrointestinal disorders but also an approach to maintain status of well-being and resist illness. Bischoff defined "gut health", based on World Health Organization (WHO) definition of health, as "a state of physical and mental well-being in the absence of gastrointestinal complaints that require the consultation of a doctor, in the absence of indications or risks of bowel disease, and in the absence of confirmed bowel disease" (Bischoff, 2011). Over the years, research gathered enough evidence to suggest that the crucial factors maintaining gut health are an intact intestinal barrier and a balanced gut microbiota. Moreover, animal and human studies have demonstrated the role of gut dysbiosis and alterations of intestinal barrier function in a vast array of pathological conditions, which include intestinal disorders such as IBD and irritable bowel syndrome (IBS), obesity and metabolic syndrome, hepatic inflammation, and pancreatitis, among others (Sánchez de Medina et al., 2014). Therefore, any intervention on these two factors has an important influence on the general health status and the prevention of multiple non-communicable diseases.

3. Inflammatory bowel diseases: the role of foods and their bioactive compounds

At the turn of the 21st century, IBD has become a global disease with increasing incidence in newly industrialized countries where societies have become more westernized. Although incidence is stabilizing in Western countries, burden remains high as prevalence surpasses 0.3% (Ng et al., 2017). The increasing incidence and prevalence and the complexity of treatment-associated costs have made IBD a very important public health problem (Høivik et al., 2013). IBD comprises a heterogeneous group of chronic inflammatory disorders of the GI tract mainly classified into Crohn´s disease (CD) and ulcerative colitis (UC) with differences in their location, distribution, type of inflammation, symptoms, and associated complications. Symptoms include inflammation of the colon and abdominal pain, diarrhea, fever, clinical signs of bowel obstruction, weakness, weight loss, and bleeding (Baumgart & Sandborn, 2007). Although the precise etiology of IBD remains unknown, it is likely influenced by a combination of genetic, immunological, and environmental factors. Indeed, growing evidence supports the role of diet, gut microbiota, intestinal barrier function, and mucosal immune response as key elements in IBD pathogenesis (Fernández-Tomé et al., 2019a). Altogether, these factors result in the production of inflammatory and oxidative stress mediators, which support an uncontrolled inflammatory response and ultimately may culminate in irreversible tissue damage (Nunes et al., 2018).

Due to the presence of food particles, pathogens, or microbiota imbalance, the GI tract may become irritated, generating an excess of ROS and compromising endogenous antioxidant defenses (Moura et al., 2015). Oxidative stress disrupts the intestinal epithelial barrier and increases intestinal permeability; the “leaky gut” further exacerbates the inflammatory process (Nunes et al., 2018). Moreover, this oxidative status affects gut microbiome leading to dysbiosis characterized by decreased community richness and diversity, increased proportions of pro-inflammatory species (*Escherichia*,

Fusobacterium) and reduced proportions of anti-inflammatory species (*Faecalibacterum*, *Roseburia*) (Winter & Baumler, 2014). Decrease of beneficial bacteria causes overproduction of ROS which further aggravates gut dysbiosis (Hu et al., 2019). Moreover, reduction of short chain fatty acids (SCFA)-producing microbial strains results in lower release of this compound, which is recognized as the major energy source for epithelial colon cells (Morgan et al., 2012). On the other hand, cytokines are cell signaling molecules generated predominantly by immune cells that have specific roles in the communication and interaction between cells, and the onset of local and systemic inflammation. Under normal conditions, the intestinal mucosa can maintain the balance between pro-inflammatory [TNF-α, interferon (IFN)-γ, IL-1, IL-6, and IL-12], and anti-inflammatory [IL-4, IL-10, and transforming growth factor beta (TGF-β)] cytokines. In IBD patients, the intestinal homeostasis and the balance between pro- and anti-inflammatory responses is disrupted, causing an increased number and activities of pro-inflammatory cytokines in the mucosa, leading to leukocyte mucosal infiltration, tissue damage and inflammation. Furthermore, the weakened epithelial barrier function and the increased intestinal permeability in IBD subjects facilitate mucosal inflammation (Al Mijan & Lim, 2018).

To date, there is no an effective therapy available to completely cure IBD. Conventional treatments based on corticosteroids and immunosuppressant drugs are incapable of targeting the pathogenic IBD mechanisms, thus they are specifically designed to induce and maintain clinical remission, and help to mitigate complications, which are nevertheless only achieved in a small proportion of the patients (Gisbert et al., 2015). Moreover, side-effects such as opportunistic infections and malignancies have been associated with the long-term use of currently approved drugs (Zenlea & Peppercorn, 2014). Therefore, the development of alternative IBD therapies based on

natural substances that are highly effective, safe, and inexpensive is in great demand (Fernández-Tomé et al., 2019b). As diet is one of the environmental factors involved in IBD etiology, a versatile dietary intervention with reduction of the intake of potential food hazards and the inclusion of food components that can target the underlying alterations of IBD is becoming a promising alternative (Durchschein et al., 2016).

Nutrients and food bioactive compounds with demonstrated effects against IBD are summarized in Figure 1. Recent reviews have described the existing evidence on the benefits exerted by these compounds (Marton et al., 2019; Sugihara et al., 2019; Gao et al., 2020; Hossen et al., 2020). According to numerous studies, dietary fiber is important in the pathogenesis of IBD. The anti-inflammatory and immunomodulatory functions of fiber are partly attributed to metabolites resulting from its fermentation by probiotic and useful bacteria at the large intestine (Nie et al., 2017). Among these metabolites, SCFA, mainly acetate, propionate, and butyrate, can reduce inflammation through decrease of pro-inflammatory cytokines (Tedelind et al., 2007; Cushing et al., 2015; Hartog et al., 2015). Moreover, increasing evidence demonstrate the role of dietary fiber, maintaining the proper function of the intestinal barrier and avoiding the entry and colonization of pathogenic bacteria (Jarmakiewicz-Czaja et al., 2020).

Dietary lipids are one of the most active nutritional substrates modulating the gut mucosal immune system. Although the high consumption of total fats may increase the risk of both UC and CD (Sugihara et al., 2019), some fat components such as polyunsaturated fatty acids (PUFAs) have been found to exert beneficial effects, thus their supplementation through the diet could be an interesting strategy for controlling IBD (Uchiyama et al., 2010; Jayarathne et al., 2017). The most interesting PUFAs are the ω-6 arachidonic acid, and the ω-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). An increasing incidence of IBD has been reported in countries where the

lifestyle has “westernizated”, with dietary changes including higher intake of ω-6 and reduced consumption of ω-3 PUFAs. Moreover, Tjonneland and coworkers observed a correlation between higher intake of ω-6 linoleic acid and an increased risk of UC (Tjonneland et al., 2009). Thus, a balanced ratio between ω-6 and ω-3 fatty acids is suggested to prevent/manage IBD. Conjugated linoleic acid (CLA), mainly found in milk, and dairy and ruminant meat products, or endogenously produced by gut bacteria, is another fat component with demonstrated beneficial effects at intestinal level through modulation of inflammation-associated biomarkers (Bassaganya-Riera et al., 2012a,b).

Among micronutrients, vitamins, mainly lipophilic vitamins A and D, have been demonstrated to exert protective effects against the pathogenesis of IBD in both cells and animal models. Moreover, increasing evidence suggests that patients affected by these disorders have low levels of these fat-soluble vitamins, thus, their specific supplementation is recommended (Uranga et al., 2016). Similarly, an adequate intake of minerals such as zinc, selenium, iron, and magnesium has been described as needed to reduce the risk of inflammatory diseases such as IBD (Jarmakiewicz-Czaja et al., 2020).

Probiotics are generally defined as food ingredients made from live microorganisms that, when ingested in an acceptable amount, promote the immune system and the gut health, resulting in beneficial effects on the host. Multiple mechanisms of action have been described for probiotic bacteria including maintenance of the natural gut microbiota, and anti-microbial, immunomodulatory, and anti-inflammatory effects (Al Mijan & Lim, 2018). These beneficial effects are exerted either by the whole microbes or through various bioactive components, including bacterial cell wall structures, microbial nucleic acids, secretions (e.g. antimicrobial proteins or other soluble proteins), metabolites, and other less soluble factors (Wan et al., 2018). Several studies have found an imbalance in the gut microbiota in IBD patients compared to non-IBD controls,

suggesting a correlation between probiotics and the course of the disease (Peterson et al., 2008). However, to date, no enough data are available to conclude if this correlation is beneficial and if so, to what degree. Only, concluding studies have demonstrated the relationship between probiotics and mucosal immune systems. These studies have shown, however, that there are some differences between various population groups and IBD patients, as well as between probiotic strains, suggesting that if there were some favorable dietary intervention, they would need to be individual to each patient's needs as opposed to a general option (Malinowski et al., 2020). Thus, more testing should be needed to provide definite results.

Phytochemicals are a wide range of natural compounds found in plants. Accumulating evidence suggest that phytochemicals have beneficial effect on several chronic diseases. At intestinal level, numerous *in vitro* and *in vivo* models have demonstrated that these compounds including phenolic compounds, glucosinolates, alkaloids, terpenoids, oligosaccharides, and quinones, among others, can influence the gut microbiota and intestinal immunity. In addition, many of these phytochemicals can modulate enzymatic activity, suppress the inflammatory transcriptional factors, inhibit oxidative stress, and reduce pro-inflammatory cytokines secretion (Hossen et al., 2020). The promising potential of these secondary plant metabolites in the prevention/management of IBD and other gastrointestinal inflammatory disorders has been recently reviewed (Uranga et al., 2016; Vezza et al., 2016; Shimizu, 2017; Zielinska et al., 2019).

4. Role of food proteins and peptides against inflammatory bowel diseases

4.1. In vitro evidence on the antioxidant, anti-inflammatory and immunomodulatory effects

In the last years, the evidence on the beneficial effects of food proteins/peptides at the GI level has notably increased. A recent review has summarized these *in vitro* effects which have been evaluated mainly using macrophages, intestinal epithelial cells, and hepatocytes (Fernández-Tomé et al., 2019a). Among proteins from animal food sources, milk proteins have become the main evaluated substrates. Casein-derived peptides have been demonstrated to be responsible for the antioxidant and anti-inflammatory activity of hydrolyzed milk products. Thus, peptides EAMAPK and AVPYPQ, released from β-casein during simulated GI digestion of Stracchino cheese, decreased ROS levels and activated Nrf2 response in H_2O_2-treated epithelial IEC-6 cells (Pepe et al., 2016). Caseinophosphopeptides have been also found to protect intestinal Caco-2 cells from oxidative damage caused by chemical agents (Laparra et al., 2008). Similarly, whey protein hydrolyzates by digestive enzymes showed antioxidant and cytokine modulatory effects in H_2O_2-stimulated Caco-2 cells (Piccolomini et al., 2012). Although the specific compounds responsible for these effects were not identified, the high content in cysteine might be the main contributor by its ability to increase the reduced glutathione levels caused by chemicals. However, in a Pronase whey protein hydrolyzate, β-lactoglobulin peptide IPAV was found to exert anti-inflammatory effects in TNF-α activated Caco-2 cells through decrease of the production of pro-inflammatory cytokines (Oyama et al., 2017).

Antioxidant and modulatory effects on cytokine production have been also found for peptides derived from egg proteins. Thus, oligophosphopeptides and their derived fragments from egg yolk phosvitin have been described to reduce IL-8 secretion and up-regulate antioxidant genes associated with oxygen and ROS metabolism, thereby contributing to the prevention of intestinal oxidative stress and the promotion of gut health (Young et al., 2011). Marine proteins are other important source of potential bioactive

peptides at GI level. A commercial fermented fish product derived from the controlled proteolytic yeast fermentation of *Merluccius productus* (pacific whiting) is claimed to be beneficial for a variety of gut conditions. In an indomethacin-induced HT29 cells model, this product inhibited intestinal caspase-3 activity, resulting in an increase of the cell proliferation and reduction of apoptosis and villus damage (Marchbank et al., 2009). Other peptides present in marine proteins hydrolyzates have also demonstrated ability, in cell models challenged by various disruptive stimuli, to attenuate NO and PGE2 production, to suppress the protein expression of inducible NO synthase (iNOS) and cyclooxygenase (COX)-2, and to act on intestinal tight junction proteins (Fernández-Tomé et al., 2019a).

Similarly to animal proteins, hydrolyzates from plant proteins such as soybean, wheat, corn, common bean, and rice have shown *in vitro* anti-inflammatory effects, although only few peptides have been identified, to date, as responsible for these activities (Oseguera-Toledo et al., 2011; Dia et al., 2014; Yin et al., 2014; Wen Chen et al., 2016). Soybean peptide VPY has been found to inhibit pro-inflammatory mediators in intestinal and immune cells which are involved in the pathogenesis of IBD (Kovacs-Nolan et al., 2012). It has been demonstrated that this peptide acts through the intestinal transporter Pept1, which levels are abnormally elevated in the colon of IBD patients, thus becoming a novel therapeutic target for this disorder (Ingersoll et al., 2012). Lunasin is another soybean peptide with potent antioxidant, anti-inflammatory, and immunomodulatory effects demonstrated in chemical and LPS-induced enterocytes, hepatocytes, and macrophages (Dia & de Mejia, 2011; Fernández-Tomé et al., 2014; García-Nebot et al., 2014). Moronta and co-workers demonstrated the immunomodulatory effects of amaranth protein hydrolyzates and their peptides SSEDIKE, IADEDPDEANDK and KPV in a bacterial flagellin-activated colonic epithelial cell model through a NF-κβ dependent

pathway (Moronta et al., 2016), suggesting the potential use of these peptides as ingredients of functional foods complementing therapies against IBD and other inflammatory disorders.

4.2. In vivo evidence on experimental models of inflammatory bowel disease (IBD)

Animal models have allowed decoding the main mechanisms involved in IBD but have also contributed to the development of new therapeutic approaches at a pre-clinical setting. In the last years, different experimental IBD models have been developed (Mizoguchi, 2012; Kiesle et al., 2015). However, models generated by chemical-induction of intestinal inflammation have been the most frequently used for the evaluation of bioactive peptides. These models have revealed that the demonstrated *in vitro* protective potential of food proteins and peptides against IBD can be translated to *in vivo* effects. The proven antioxidant, anti-inflammatory and immunomodulatory activities of food derived hydrolyzates and peptides in different IBD-like animal models are summarized in Table 1.

Oral administration of dextran sulfate sodium (DSS) results in a direct toxic effect on the colonic epithelium with loss of its barrier function, thereby allowing the entry of luminal organisms into the lamina propria. Thus, this model mimics some features of UC such as mucin depletion, increased permeability, neutrophilic infiltration, crypt abscess, and superficial mucosal erosions (Kiesler et al., 2015; Valatas et al., 2015). DSS-induced model has been used to evaluate the protective effects of hydrolyzates and peptides from both animal and plant food sources (Table 1). Inclusion of these digests/peptides in the diet has been demonstrated to ameliorate the clinical symptoms (weight loss, mucosal and submucosal inflammation, colon length shortening and colonic damage) through down-regulation of the colonic expression of pro-inflammatory biomarkers (such as IL-1β,

TNF-α, IL-6, IFN-γ and IL-17A, among others), and up-regulation of anti-inflammatory biomarkers (IL-10 and TGF-β) and antioxidant enzymes activity (Zhao et al., 2017; Liu et al., 2018a; Ma et al., 2019a, b; Deng et al., 2020a, b). Moreover, the protective effects have been also linked to the restoration of bacterial microbiota composition after the inflammation-induced dysbiosis (Wada et al., 2013; Liu et al., 2018a).

Another animal model of IBD involves the intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) resulting in a Th1-mediated colitis characterized by infiltration of CD4+ T cells, neutrophils and macrophages followed by crypt destruction which is associated with weight loss, severe diarrhoea, rectal prolapse, and bleeding, resembling all together some of the pathologic characteristics of CD (Kiesler et al., 2015; Valatas et al., 2015). Some food protein compounds have been found to attenuate the clinical symptoms, activity index, histological scores, and immune mediators of intestinal inflammation in TNBS-induced animal models (Table 1). This is the case of caseinomacropeptide that after its oral administration for 9 days, reduced inflammatory injury through reduction of mieloperoxidase (MOP), alkaline phosphatase, iNOS, and pro-inflammatory cytokines (Requena et al., 2008). More recently, protective effects have also been found for a Alcalase® hydrolyzate of flaxseed proteins (Silva et al., 2018). Peptides present in the hydrolyzate were demonstrated to decrease T cell proliferation, expansion of TH1 and TH17 cells, and pro-inflammatory biomarkers.

Although the current available IBD animal models allow studying multiple defects in the intestinal immune system, none of them can fully mimic the complexity of human IBD. In addition, the genetic heterogeneity and disease phenotypes of IBD patients in clinical trials are not comparable to homogeneous populations of animal models. Thus, gut health allegations concerning bioactive peptides cannot be drawn just from *in vitro* data or animal models (Bouglé & Bouhallab, 2017), and clinical trials are

required before peptides can be translated into the human context. Together probiotics, prebiotics, phytochemicals, fatty acids, and lipophilic vitamins, peptides and amino acids are among the most investigated food compounds for their potential impact on human IBD (Larussa et al., 2017). However, the *in vivo* evidence is still limited (Table 2). First studies demonstrated an induction of remission in active CD patients after nasogastric administration of an oligopeptide-based diet although the effects were attributed to the constituent amino acids (Mansfield et al., 1994). However, recently, an anti-inflammatory effect was observed in patients affected by this intestinal disorder after oral ingestion of whey and soy protein, suggesting that peptides released during gastrointestinal digestion were the main responsible for these benefits (Machado et al., 2015). The peptides contained in the BBI concentrate were also described as the contributors on the reduction of the disease activity index and the beneficial effects in the rates of remission and clinical response of UC patients (Lichtenstein et al. 2008). In a very recent study, reduction of gastrointestinal symptoms was found when a casein hydrolyzate was administered to patients affected by IBS for 10 days (Laatikainen et al., 2020). Because of these preliminary results, further research is needed to confirm the protective effects of food proteins/peptides against IBD and other inflammatory intestinal disorders.

5. Effects of food peptides on gut microbiota

The gut microbiota is relatively stable in healthy adult human populations with 60% of strains remaining over the course of 5 years (Faith et al, 2013). A total of 2172 species have been isolated from human beings, classified into 12 different phyla, of which 93.5% belonged to Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes (Thursby & Juge, 2017). An analysis of gut microbial communities proposed three predominant variants, or "enterotypes", dominated by *Bacteroides*, *Prevotella* and

Ruminococcus, respectively (Arumugam et al., 2011). The first enterotype, high in *Bacteroides* and low in *Prevotella,* is found in individuals on a long-term Western diet rich in animal proteins, choline, and saturated fats (Wu et al., 2011). The second enterotype, high in *Prevotella* and low in *Bacteroides*, is associated with a plant-based diet rich in fiber, simple sugars, and plant-derived compounds. A third potential enterotype was found to have a slightly higher population of genus *Ruminococcus* within the phylum Firmicutes. However, the existence and formation of these enterotypes is controversial (Jeffery et al., 2012).

When intestinal inflammation or IBD occurs, in addition to the inflammatory process, the gut microbial community also changes, with the decrease of Firmicutes (particularly *Clostridium* groups) and the increase of *Bacteroides, Lactobacillus, Eubacterium*, and *Proteobacteria* (Nagalingam & Lynch, 2012). Thus, patients affected with CD display mucosal dysbiosis characterized by reduced diversity of core microbiota, presence of Proteobacteria such as adherent-invasive *Escherichia coli*, *Campylobacter concisus* and enterohepatic *Helicobacter*, and low abundance of *Faecalibacterium prausnitzii* which may serve as a biomarker to assist in the diagnostics of this gut disease (Lopez-Siles et al., 2017). Moreover, *F. prausnitzii* has been demonstrated to protect against DNBS-colitis in mice through production of anti-inflammatory peptides which were additionally able to inhibit activation of NF-κB pathway in intestinal epithelial cells (Quevrain et al., 2016). Also, gut enrichment with *Ruminococcus* is associated with IBS (Rajilic-Stojanovic et al., 2015), and transient blooms of pro-inflammatory *Ruminococcus* have been associated with active flare-ups in IBD (Hall et al., 2017). High levels of *E. coli* have also been associated with IBD and cancer (Veziant et al., 2016). Proliferation of this specie and other *Enterobacteriaceae* has been associated with inflammation,

colitogenic effects and promotion of the growth of populations playing a role in carcinogenesis (Amaretti et al., 2020).

Different therapeutic strategies for targeting dysbiosis in IBD through modulation of the altered gut microbiota, such as probiotics and fecal microbiota transplantation are currently being explored (Nishida et al., 2018). The impact of carbohydrates, proteins and fats on the composition of gut microbiota is also known, thus strategies involving modifications in the intake of these macronutrients are being studied to modulate altered microbiota in IBD and other intestinal inflammatory disorders (David et al., 2013; Oliphant and Allen-Vercoe, 2019). Moreover, the inclusion into the diet of some foods such as red seaweeds has been found to modulate the gut microbiota and to enhance the intestinal mucosal barrier function in a chicken model (Kulshershtha et al., 2014). The microbiota modifications were accompanied by higher SCFA concentrations, pointing to an overall prebiotic effect of the seaweeds. In a randomized controlled trial with CD patients, the intake of fermented dairy product kefir resulted in a significant change in *Lactobacillus* population whereas no changes were observed in UC patients (Yilmaz et al., 2019). However, the bioactive compounds responsible for the observed effects were not identified. In the case of bioactive peptides, some data associated them with anti-inflammatory and gut microbiota modulatory effects in IBD models (Table 3). In a colon colitis mouse model, wheat gluten-derived peptide pyroglutamyl leucine was found to normalize the population of Bacteroidetes and Firmicutes (Wada et al., 2013). Other peptides such as IRW and IQW, from egg albumin and transferring, respectively, have been also demonstrated to increase the abundance of Firmicutes and Actinobacteria species, and to reduce the proportions of Bacteroidetes and Proteobacteria species inhibiting colonic inflammation on DSS- and *Citrobacter rodentium*-induced colitis in mice, respectively (Liu et al., 2018a, Ma et al., 2019a). Dietary di-peptide GQ was also

found to change gut microbiota in a beneficial manner through increasing alpha diversity, bacterial loading, abundance of anaerobes and fiber-degrading bacteria (Phylum Fibrobacteres), and SCFA in the gut (Yan et al., 2020).

6. Future prospects

Some dietary patterns have been associated with the incidence of human GI disorders. Diet is one of the main environmental players affecting GI disorders as dietary compounds pass throughout the intestinal lumen and can modulate the function of the epithelial cells, mucosal immune system and the gut microbiota. Indeed, maintenance of gut homeostasis, which includes the integrity of the mucosal and a balanced microbiota, is essential to avoid not only locally IBD but also some other chronic systemic disorders of the organism. Hence, dietary intervention over these factors has reached a notable and increasing impact in the literature over the last years. Regarding food proteins and peptides, they have shown a variety of complementary mechanisms on gut barrier protection by several *in vitro* (cell cultures) and, to a lower extent, *in vivo* models. Evidence on bioactivity at GI level is promising despite, beyond that demonstrated in animal models, just a few preliminary data have been translated into the human setting. This, in combination with the high variability between studies, hinders the interpretation of the evidence achieved so far but encourages the need of more robust information as well as the design of future studies that validate previous findings in healthy subjects and IBD patients.

Along with this, since the gut microbiota and microbial metabolites are promising mechanisms for bioactive peptides to act against intestinal inflammation, further studies exploring the interaction between peptides and microbial communities should be needed. Studies found on this topic have showed some preliminary data but are still in its infancy.

However, to address whether microbiota changes are behind the cause and/or consequence of intestinal disorders still represents an unresolved matter of concern under investigation. Moreover, integration of the multi-layered data of these studies and its appropriate interpretation is a real challenge. Well-controlled human intervention trials in combination with the application of "omic" approaches (metagenomics, transcriptomic, metaproteomics and metabolomics) and bioinformatics tools should be used and, fortunately, may allow at advancing the knowledge and understanding of the relationship between food peptides and gut microbiota on IBD and other intestinal inflammatory disorders. The development of food peptide-based functional compounds for GI disorders is expected to take advantage of these investigations in the near future.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by the Spanish Ministry of Economy and Competitiveness (AGL2015-66886-R), and CSIC (PID2019-103919RB-100). S.F.-T is currently funded by the Instituto de Salud Carlos III (Sara Borrell fellowship CD17/00014).