Novel Perspectives in Celiac Disease Therapy

Yolanda Sanz*

Microbial Ecophysiology and Nutrition Group. Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC). PO Box 73, 46100 Burjassot, Valencia, Spain

Abstract: Currently the only treatment for celiac disease is adherence to a strict gluten-free diet; however, compliance with this diet is complex and other alternatives are called for. Herein, we review recent developments in the design of novel therapeutic strategies to counteract the pathogenic effects of the interactions between gluten peptides and their specific biological targets.

Key Words: Celiac disease, gluten, immunity, permeability, therapy, intestinal microbiota, probiotics.

INTRODUCTION

Celiac disease (CD) is a chronic inflammatory disorder of the small intestine triggered by the ingestion of cereal gluten proteins in genetically susceptible individuals. Intestinal tissue lesion of active CD patients is characterized by villi atrophy, crypt hyperplasia, and inflammatory cell infiltration [1]. The disease is characterized by nutrient malabsorption and gastrointestinal symptoms but it can also cause extra-intestinal symptoms or apparently have no clinical expression [2].

The pathogenesis of CD involves interactions between genetic, immunological and environmental factors (Fig. (**1**)). Susceptibility to CD is strongly associated with the Human Leukocyte Antigen (HLA) genes of the major histocompatibility complex. Around 95% of patients express the alleles coding for HLA-DQ2 and HLA-DQ8 heterodimer molecules [3]. HLA-DQ2/DQ8 molecules of antigen-presenting cells bind and present specific gluten peptides (e.g. a 33-mer of the alpha-gliadins) to lamina propria CD4+ T cells, triggering a Th1 biased immune response, with production of mainly interferon gamma (IFN-γ) [4]. The deamidation of gluten peptides by tissue transglutaminase (tTG) promotes their binding to HLA-DQ2 and HLA-DQ8 molecules and increases their immunogenicity [5]. Moreover, other gluten peptides (e.g. 19-mer) trigger an innate immune response characterized by interleukin (IL)-15 production by epithelial and dendritic cells and expansion of intraepithelial TCRγ/δ+ and CD+8 TCRα/β+ lymphocytes, which are cytotoxic for epithelial cells and contribute to cell killing [6, 7]. The disease is also associated with increases in epithelial permeability due to tight junction dysfunction as reported in other autoimmune diseases. In particular, up-regulation of zonulin expression, a molecule that reversibly modulates tight-junction opening and paracellular permeability, is involved in CD [8].

*Address correspondence to this author at the Microbial Ecophysiology and Nutrition Group. Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC). PO Box 73, 46100 Burjassot, Valencia. Spain; Tel: 34 963900022; Fax: 34 963636301;
E-mail: yolsanz@iata.csic.es

Dietary gluten is the major environmental factor responsible for symptoms and signs of CD. The principal toxic components of gluten are proline- and glutamine-rich peptides from wheat, rye and barley, which are resistant to complete proteolytic digestion and susceptible to deamidation by tTG. Moreover, given the role of gut microbes in immunity and gut barrier functions, one cannot disregard the possible role played by the microbiota in the risk and pathogenesis of CD. In fact, imbalances in the composition of the intestinal microbiota of CD patients, as well as changes in metabolites related to the activity of the gut microbiota, have been associated with the disease recently [9-11]. Some epidemiological studies also indicate that the frequency of microbial infections may increase the risk of CD autoimmunity in childhood [12]. However, another recent study suggests that lower levels of prosperity and hygiene may guard against CD, which would explain the lower prevalence of tTG antibodies and CD in the Russian Karelia population as compared to the Finnish population [13] Most probably, both theories are over simplifications of the complex process whereby oral tolerance is developed, which might depend on the type and amount of environmental stimuli and timing of exposure as well as on the particular genotype.

CD is the most common lifelong enteropathy, with a prevalence of 0.5-2.0% in the general population [14]. Nevertheless, the only treatment of this disorder is lifelong exclusion of gluten from the diet. Complying with this dietary recommendation is difficult and affects the patient's quality of life. Therefore, there is a need for therapeutic alternatives. The following sections review the state of art of novel therapeutic strategies being investigated to tackle CD, intended to interfere with the interaction between gluten peptides and their specific biological targets within the gastrointestinal tract. A chart showing these therapeutic targets is shown in Fig. (**1**).

ENZYMATIC DEGRADATION OF GLUTEN PEPTIDES

The principal toxic components of gluten are proline- and glutamine-rich peptides that are resistant to proteolysis by gastric, pancreatic and intestinal brush border membrane enzymes [15]. Therefore, they can accumulate in the small

1389-5575/09 $55.00+.00

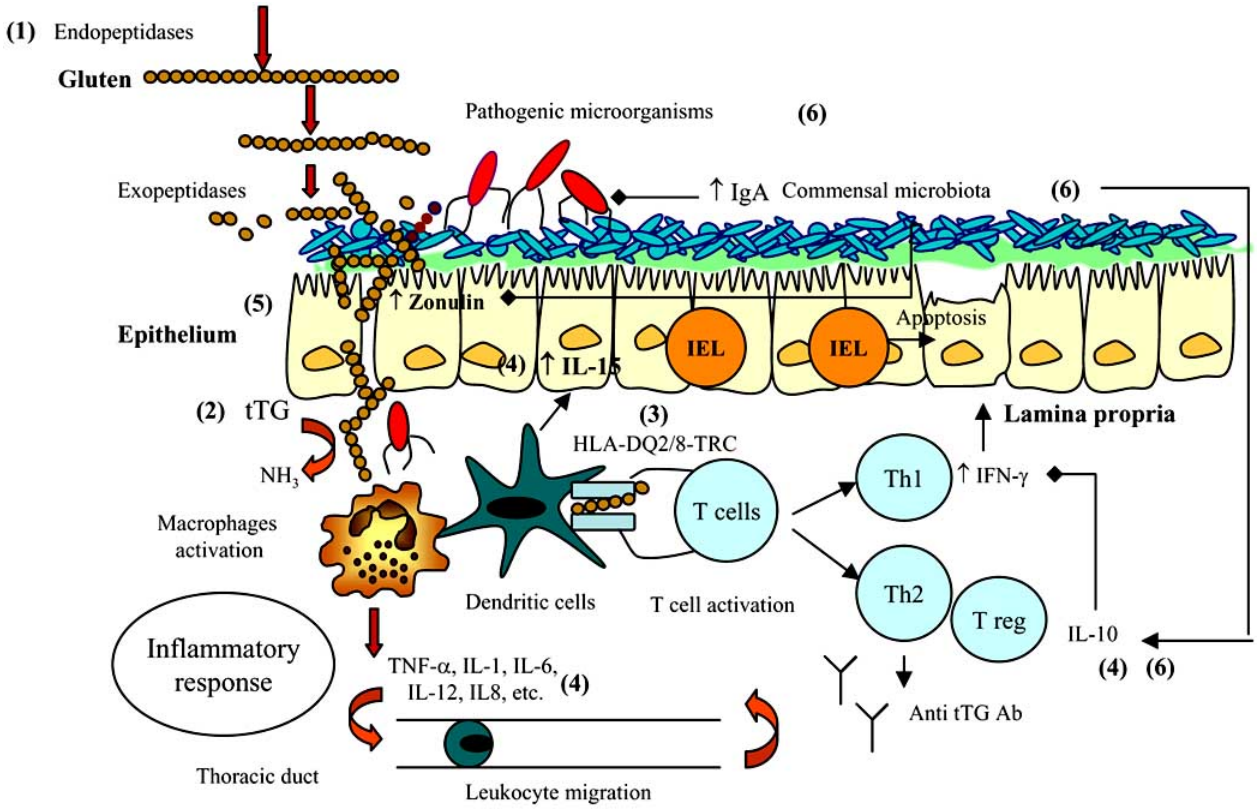

Fig. (1). Schematic representation of the pathogenic mechanism of gluten peptides in CD, and the biological targets for developing alternative therapies to the gluten-free diet. (1) Endopeptidases and exopeptidases able to degrade gluten peptides resistant to digestive enzymes before activating an aberrant immune response in the small intestine; (2) Inhibitors of tTG that prevent the conversion of glutamine residues into glutamic acid, with higher affinity for HLA-DQ molecules and immunogenicity; (3) Inhibitors of gluten peptide antigen presentation by HLA-DQ molecules that prevent activation and clonal expansion of T-cell subsets involved in the humoral response with production of disease–specific antibodies (e.g. anti-tTG antibodies) and in the cell-mediated Th1 immune response with release of pro-inflammatory cytokines (IFN-γ and TNF-α); (4) Anti-inflammatory cytokines like IL10 that counteract the Th1-biased immune response elicited by gluten peptides; anti pro-inflammatory cytokine drugs that block the action of the key cytokines involved in CD pathogenesis (IFN-γ, TNF-α, and IL-15); and (4) inhibitors of cell-adhesion molecules that prevent the migration of T cells to the lamina propria and their interaction with peptides presented *via* HLA-DQ molecules of antigen presenting cells; (5) Inhibitors of molecules involved in the increase of paracellular permeability associated with CD (e.g. inhibitors of the zonulin receptor) triggered by gluten peptides and pro-inflammatory cytokines; (6) Commensal bacteria and probiotics that compete with pro-inflammatory pathogenic microorganisms and contribute to the host's defense mechanisms by promoting secretory-IgA production, reducing paracellular permeability and regulating aberrant immune responses by inducing IL-10 production and T regulatory cells.

intestine eliciting a pathogenic immune response in genetically susceptible individuals. This link between digestive enzyme resistance and gluten toxicity has triggered a search for enzymes able to hydrolyze gluten peptides, thereby neutralizing their adverse effects.

In this context, proline-specific peptidases, including prolyl endopeptidases and proline specific exopeptidases (e.g., proline iminopeptidases, X-prolyl-dipeptidyl peptidases, prolinases and prolidases), are ideal candidates for use in gluten toxicity reduction [16] (Table **1**). Unlike most peptidases, prolyl endopeptidases are proteases with the unique ability to hydrolyze the peptide bonds on the carboxyl side of a proline residue, generating smaller peptides that are then suitable substrates for exopeptidases (Table **1**, Fig. (**1**)). These enzymes are widely distributed in bacteria, fungi, animals and plants. However, human prolyl endopeptidase locates intracellularly in the epithelium, similarly to dipeptidyl peptidase IV, so it can hardly contribute to gluten peptide digestion. In addition, the mammalian prolyl endopeptidase is unable to digest 33-mer peptide, which, conversely, is a prolyl endopeptidase inhibitor; therefore other peptidases are needed to eliminate noxious gliadin-derived peptides [17].

Initially, most studies aiming to find a suitable enzyme for CD therapy have used the prolyl endopeptidases of *Flavobacterium meningosepticum, Sphingomonas capsulate* and *Myxococcus xanthus*, which are members of the serine peptidase family S9A (Table **1**). These enzymes are similarly able to break down gluten peptide sequences and reduce their immune and toxic effects in *in vitro* and *ex-vivo* model systems [15, 18]. A clinical study on CD patients has also demonstrated that the pre-treatment of gluten with the *F. meningosepticum* enzyme prevented nutrient malabsorption from taking place in the majority of patients [19]. However, the abovementioned enzymes are not active at low stomach pH and are susceptible to pepsin degradation. Thus, they would be unable to work optimally *in vivo,* being inactive once

Table 1. Main Biochemical Properties of Microbial Proline-Specific Peptidases

Enzyme type	Origin	EC number	Catalytic type	Specificity	Family
Oligopeptidases				Xn-⇓-X-Yn	
Prolyl oligopeptidase	*Flavobacterium meningosepticum*	3.4.21.26	Serine	Xn-⇓-Pro-Yn	S9A
	Sphingomonas capsulate				
	Myxococcus xanthus				
	Aspergillus niger		Serine	Xn-⇓-Pro-Yn	S28
Endopeptidase (PepO2/PepO3)	*Lactobacillus helveticus*		Metallo	Xn-⇓-Pro-Yn	
Dipeptidases				X-⇓-Y	
Prolidase (PepQ)	*Lactobacillus delbrueckii*	3.4.13.19	Metallo	Pro-⇓-Y	M24B
Prolinase (PepR)	*Lactobacillus helveticus*		Serine	X-⇓-Pro	
	Lactobacillus rhamnosus				
Dipeptidyl-peptidases				X-Y-⇓-$(Z)_n$	
X-Pro-dipeptidyl peptidase (PepX)	*Lactococcus lactis*	3.4.14.11	Serine	X-Pro-⇓-$(Z)_n$	S15
	Lactobacillus delbrueckii				
	Lactobacillus helveticus				
Aminopeptidases				X-⇓-(Y)n	
Proline-iminopeptidase (PepI)	*Lactobacillus delbrueckii*	3.4.11.5	Serine	Pro-⇓-(Y)n	S33
	Lactobacillus helveticus				

reaching the small intestine where gluten causes the most damage.

Further, an acid prolyl endopeptidase from *Aspergillus niger,* initially used to reduce bitterness of casein peptides, was evaluated for the same purpose [18]. This enzyme is a member of the serine peptidase family S28, which shares more sequence homology with the lysosomal Pro-X carboxypeptidase than with prolyl oligopeptidases, works optimally at pH 4-5, remains stable at pH 2, and is resistant to pepsin digestion (Table **1**). The enzyme rapidly degraded different T-cell stimulatory peptides, as well as intact gluten molecules *in vitro*, leading to the destruction of the T-cell epitopes as demonstrated by T-cell proliferation assays and immunologic tests [18]. The efficacy of this enzyme in degrading gluten was further evaluated in a dynamic human gastrointestinal model system fed with either a slice of bread or a standard fast food menu with and without co-administration of the enzyme [20]. The enzyme accelerated the degradation of gluten in the stomach compartment to such an extent that hardly any gluten reached the duodenal compartment.

In addition, the ability of a new combination of enzymes with complementary activities to detoxify gluten has been evaluated using *in vitro* and *in vivo* (rat) experimental systems [21]. This combination consists of a glutamine-specific endopeptidase from barley, which hydrolyzes intact gliadin polypeptides under acidic conditions but it is unable to cleave some of the inflammatory peptides (e.g. 33-mer from α-gliadin), and a duodenally active prolyl endopeptidase from *Sphingomonas capsulate* able to degrade the 33-mer for instance. The first enzyme extensively hydrolyzes complex gluten proteins of bread, whereas the prolyl endopeptidase rapidly detoxifies the residual oligopeptide products of the first digestion. Thus, it has been suggested that the administration of this combination of enzymes could increase the safe threshold of ingested gluten, thereby ameliorating the burden of a highly restricted diet for patients with CD [21].

Similar enzymes have been detected in lactic acid bacteria [16, 22], although the post-prolyl endopeptidases identified in these bacteria are metallo peptidases (Table **1**). In addition, lactobacilli exopeptidases (amino- and dipeptidases), which hydrolyze peptide bonds at the N-terminus of polypeptides and show specificity for proline-containing peptides (e.g. PepI, PepX, PepR and PepQ), could contribute to the progress of the proteolytic chain, thereby leading to the generation of innocuous free amino acids and very small peptides. Some of these lactic acid bacteria have been used to detoxify gluten-containing products before they were ingested [16, 23]. In particular, *Lactobacillus sanfranciscensis* LS40 and LS41 and *Lactobacillus plantarum* CF1 were selected on the basis of their ability to hydrolyze gluten peptides during the fermentation process of cereal-based products. These lactobacilli have been shown to hydrolyze and

reduce the toxicity of low amounts of gluten present as contaminants in gluten-free products and to enhance the nutritional properties of bread [23]. The possible use of these enzymes to detoxify gluten during the gastrointestinal passage has been proposed but has not been evaluated yet [16].

Overall the findings suggest that the co-administration of enzyme(s) with a gluten-containing meal could reduce gluten toxicity, and offer patients the possibility of occasionally abandoning their strict gluten-free diet [20, 21]. The *in vivo* efficacy and the safety of the therapeutic enzyme(s) regarding allergen properties, systemic effects, and possible alterations of intestinal physiology have yet to be evaluated.

TISSUE TRANSGLUTAMINASE (tTG) INHIBITORS

tTG plays a major role in the pathogenesis of CD as it is both the target of specific auto antibodies and promotes the gluten-specific T-cell response [5]. tTG is found just below the epithelium and in the brush border and it catalyzes the conversion of specific glutamine residues of gliadin peptides into glutamate (Figs. (**1**) and (**2**)). Thus, tTG generates negatively-charged amino-acid residues that bind to HLA-DQ2 or DQ8 molecules with higher affinity, promoting the pro-inflammatory T-cell response [5, 24]. tTG can also cross-link gluten peptides with matrix proteins, thereby retaining gluten in the tissue environment and generating complexes that induce an immune response to additional auto antigens. tTG antibodies may also play a direct pathogenic role in CD by activating monocytes *via* Toll-like 4 receptor binding [25] and by disturbing several steps of angiogenesis [26]. As a consequence tTG inhibitors, including reversible competitive inhibitors and irreversible inhibitors, have been evaluated as potential therapeutic agents for CD [27]. Competitive inhibitors, such as cystamine and mono-dansyl-cadaverine, act by competing with natural amine substrates in the transamidation reaction (Fig. (**2**)). Although tTG remains enzymatically active in the presence of these inhibitors, initial studies have shown that blocking the endogenous tTG activity in the celiac biopsies with cystamine, reduced the proliferative response of T-cell lines to deamidated gluten peptides compared to non-cystamine treated controls [24].

Irreversible tTG inhibitors have a more specific mechanism of action, preventing substrate binding by covalent modification of the enzyme. Most of them are designed to target the active site cysteine using chemical functional groups that are reactive in the presence of a nucleophilic atom but form relatively stable chemical bonds after reacting. One of the irreversible inhibitors studied for CD treatment is the 2-[(2-oxopropyl)thio]imidazolium derivative L682777. This compound was able to prevent the *in situ* cross linking of gluten peptides to endogenous proteins of tissue sections as well as the T-cell activation induced by gluten peptides in celiac small intestinal biopsies [28]. However, this compound is also a specific inhibitor of Factor XIIIa, and therefore unsuitable for use in animals and humans. Alternatively, the halo-dihydroisoxazole compound KCC009 has shown good oral bioavailability, short serum half-life, and efficient tTG inhibitory activity in small intestinal tissue and low toxicity in mice after oral administration [29].

As yet the effects of such drugs have not been evaluated in clinical trials and their efficacy is questioned because some gluten T-cell epitopes are also recognized without needing to be modified by tTG. In addition, there is concern about the possible side-effects of tTG inhibitors as a treatment for CD because this enzyme is found in most bodily tissues and has a wide range of biological roles, including wound and tissue repair and macrophage phagocytosis [30]. It is believed that the optimal therapeutic tTG inhibitor for CD should be selective and act only locally at the gut mucosa level. Further advances, also thought to be crucial to developing future therapeutic strategies, concern the discovery of the mechanisms by which tTG, which is normally inactive in the intestine, initiates the deamidation of gluten.

INTERFERENCE WITH GLUTEN PEPTIDE PRESENTATION BY HLA-DQ MOLECULES

CD is closely associated with HLA-DQ2/DQ8 molecules, which are involved in the disease pathogenesis by presenting gluten peptides to T cells (Fig. (**1**)). Therefore, blocking the peptide-binding sites of DQ2/DQ8 molecules has been considered as a way to treat CD. The possible therapeutic strategies developed to interfere with peptide antigen presentation so far comprise: (i) modified HLA-DQ peptide ligands (termed HLA agonists), which can induce anergy in T-cells, and HLA-DQ blockers that act by out-competing the HLA binding of the agonist peptide [31].

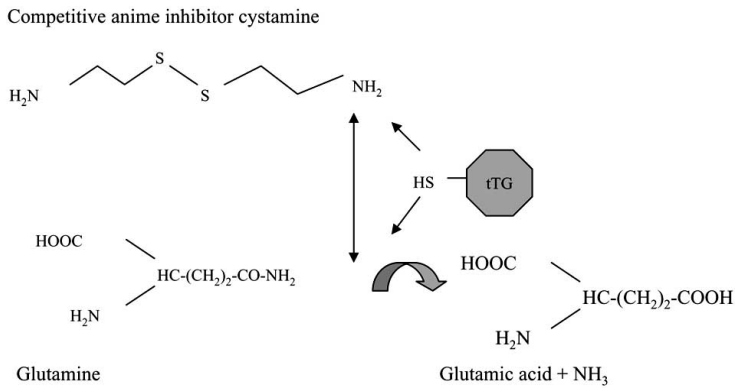

Fig. (2). Schematic representation of the peptide modification carried out by tTG and its possible inhibition. tTG catalyzes deamidation from the side chain glutamine into glutamic acid; this reaction can be inhibited by competitive inhibitors, such as cystamine, which have primary amines that compete with the natural substrate for tTG binding.

Peptides that are analogs of gliadin T-cell epitopes, which act as antagonists and can down-regulate the pathogenic immune response of CD, have been obtained artificially by amino acid substitution of gliadin T-cell stimulatory sequences [32]. In addition, a decapeptide (QQPQDAVQPF) obtained from an alcohol-soluble protein fraction of durum wheat was shown to exert an antagonistic effect *in vitro* against gliadin toxicity by reducing lymphocyte activation triggered in peripheral blood mononuclear cells by exposure to the peptic-tryptic digest of bread wheat gliadin and peptide 62-75 from alpha-gliadin [32]. An aldehyde-functionalized gluten peptide analogue has also been designed, which acts as both a tight-binding HLA-DQ2 ligand and a high-affinity, reversible inhibitor of tTG [33].

The potential use of cyclic peptides and dimeric peptides as blockers to prevent DQ2 mediated antigen recognition by T-cell receptors of CD patient mucosa has also been evaluated recently [31]. Cyclic peptides containing the DQ2-alphaI gliadin epitope LQPFPQPELPY were synthesized by introducing flanking cysteine residues cross linked *via* a disulfide bond. Cyclic peptides were also prepared with stable polyethylene glycol bridges across internal lysine residues of modified antigenic peptides such as KQPFPEKELPY and LQLQPFPQPEKPYPQPEKPY. Dimeric peptides were obtained by dimerizating the peptide LQLQPFPQPEKPYPQPELPY through the lysine side chains (Fig. (**3**)). One dimeric peptide analogue with an intermediate linker length was found to be especially effective at inhibiting DQ2-mediated antigen presentation [31].

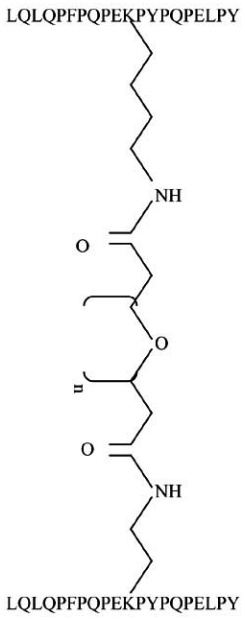

Fig. (3). Basic structure of dimeric peptides evaluated as HLA-DQ2 blockers to prevent DQ2 mediated antigen recognition by T cells (adapted from [31]).

These strategies had previously been explored as therapies for other autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and type I diabetes, but were unsuccessful due to difficulties in achieving effective drug delivery. In contrast to the organs affected by other autoimmune diseases, the small intestine affected by CD is readily accessible *via* oral administration of a therapeutic compound [31]. In particular, the modified peptides suitable to treat CD should presumably reach the small intestine in an active form, as do their analogous dietary gluten peptides, after oral administration. Therefore, although the development of this strategy is still in its infancy and its *in vivo* efficacy has not been evaluated, the use of HLA-DQ blocker peptides seems promising as an alternative to CD treatment.

CYTOKINE AND ADHESION MOLECULE REGULATION

Anti-inflammatory cytokines and antagonists of pro-inflammatory cytokines have been considered as possible therapeutic drugs for chronic inflammatory and autoimmune disorders, including CD. The use of recombinant IL-10 has been proposed as a therapy because this cytokine down-regulates activation of Th-cell subsets and particularly counteracts the Th1-biased immune response of CD and Crohn's disease (Fig. (**1**)). IL-10 also inhibits macrophage inflammatory cytokine production (e.g. TNF-α) and T-cell associated macrophage activity. IL-10 administration has exerted beneficial therapeutic effects in several murine models of colitis and in Crohn's disease patients by intravenous administration [34]. In the context of CD, recombinant human IL-10 has been shown to suppress Th1-mediated immune responses to gliadin in *ex-vivo* cultured treated and untreated celiac intestinal mucosa *via* down regulation of antigen presentation, reduction of T-cell infiltration and activation, and induction of hyporesponsiveness in gliadin-specific T cells [35]. However, the administration of recombinant IL-10 subcutaneously to refractory CD patients exerted little effect in a pilot study [36].

Alternatively, the administration of inhibitors of cytokines playing a key pathologic role in CD, such as IFN-γ and IL-15, has been considered as a strategy to treat the disease (Fig. (**1**)). IFN-γ is the main Th1-type cytokine produced by gluten-reactive T cells. IFN-γ also enhances the production of TNF-α, which altogether contributes to increasing intestinal permeability and damaging the intestinal mucosa [4, 35]. The efficacy of anti- IFN-γ and anti TNF-α drugs has been investigated in Crohn's disease patients [37]. In particular, antagonists of TNF-α, such as infliximab, certolizumab pegol and adalimumab, have afforded important therapeutic benefits to these patients, although other alternatives are also being studied, including anti IFN-γ drugs [38]. This kind of therapy could also be applied to CD according to the beneficial effects detected *in vitro* and in *ex-vivo* model systems using IFN-γ inhibitors [39]. IL-15 also plays a key role in the innate immune response elicited by gluten peptides, characterized by expansion of intraepithelial lymphocytes, which are cytotoxic for epithelial cells [6]. The efficacy of anti-IL-15 antibodies (HuMax IL-15) and an antagonistic mutant IL-15-Fc protein that targets the IL-15 receptor has been investigated in preclinical and phase II clinical trials to treat rheumatoid arthritis [40, 41]. These strategies are also presumably applicable to CD. In general, therapies based on the administration of either cytokines or cytokine inhibitors are well tolerated, although they may have some drawbacks related to organ-specific delivery and their possible systemic side-effects in the long-term [38].

In addition to cytokine-based strategies, blockage of leukocyte migration from the bloodstream to inflamed sites by the use of cell adhesion molecule inhibitors is being investigated (Fig. (**1**)). Antagonist antibodies of alpha4 integrin adhesion molecule, expressed by most leukocytes, like Natalizumab, constitute one of the new effective treatments for patients with inflammatory bowel diseases and multiple sclerosis [42, 43]. In addition, the antagonist MLN02, which targets the adhesion molecule integrin-α4β7 expressed by intestinal T cells, has been tested in phase II clinical trials for the treatment of inflammatory bowel diseases [44]. CCX282-B is also an antagonist of the chemokine receptor CCR9, which is a highly specific receptor expressed by T cells migrating to the digestive tract and recruited in the epithelium of the small intestine. This compound has also shown a certain degree of clinical efficacy in inflammatory bowel diseases [45]. It can be speculated that this compound could also interfere with the action of the HLA-DQ-restricted T cells recognizing gluten peptides in the lamina-propria. The possible side-effects of these compounds include increased susceptibility to gastrointestinal infections and disturbances in oral tolerance to food proteins because they act by preventing migration of T cells to the lamina propria in the event of danger.

INCREASED GUT PERMEABILITY INHIBITORS

Cytoskeleton and tight-junction proteins regulate the permeability of the intestinal epithelium, preventing the entry of harmful bacteria and dietary antigens. Breakdown of this barrier is usually associated with inflammation due to increased traffic of molecules from the intestinal lumen to the submucosa with excessive activation of the mucosal immune system. In CD paracellular permeability is enhanced and the integrity of the tight junction system is compromised, constituting one of the possible early events in the pathogenesis of this disorder [46]. In particular, the expression of zonulin, a protein that modulates intercellular tight junctions, was increased in intestinal tissues of CD patients [8]. Moreover, biopsy specimens of CD patients displayed a stronger response to gliadin when compared with non-celiac controls, characterized by an increased and persistent release of zonulin and significant increase in permeability [47]. Exposure of intestinal epithelial cells to gliadin induced the release of zonulin in the cell medium with subsequent binding to the surface of zonulin receptor-positive cells, rearrangement of the cell cytoskeleton, loss of occludin-ZO1 protein-protein interaction, and increased monolayer permeability [47]. Gliadin peptides have been shown to bind specifically to the chemokine receptor CXCR3, thereby leading to a MyD88-dependent zonulin release [48]. This evidence has led to the proposal of CD therapies that strengthen the epithelial barrier function and limit the access of gluten peptides to the submucosa. The administration of AT-1001, a zonulin receptor antagonist, led to a significant rate of protection of CD patients exposed to wheat proteins [49]. AT-1001 was shown to be well tolerated and appeared to reduce intestinal barrier dysfunction, pro-inflammatory cytokine production (IFN-γ), and gastrointestinal symptoms in CD patients. Larger-scale trials to verify the degree of efficacy of AT-1001 for the treatment of CD are underway.

MODULATION OF THE INTESTINAL MICROBIOTA

CD is also characterized by a breakdown in the balance between beneficial and potentially harmful bacteria in the intestine, termed dysbiosis, which may contribute to the pathogenesis of this disorder [10, 11]. It has been suggested that a dominant Th1-type cytokine profile may be triggered by harmful microorganisms that stimulate IFN-γ and TNF-α production and activate T-cell macrophage associated activity (Fig. (**1**)) [50]. These pro-inflammatory cytokines (IFN-γ and TNF-α) as well as IL-15 could contribute to increasing epithelial permeability thereby favoring the access of higher antigen loads (gliadin and microbial) to the submucosa [51]. Thus, infections or intestinal dysbiosis could facilitate an increase in permeability, spreading of gluten-reactive T cells and loss of gluten tolerance in predisposed individuals [4]. On this basis, it can be hypothesized that the administration of probiotics, which modulate the composition and functions of the intestinal microbiota, could be an adjuvant strategy in the management of CD. Specific probiotic strains have been shown to protect against leakage of tight-junctions detected in infections, stress, and inflammatory conditions [52, 53]. In the context of CD, a *Bifidobacterium lactis* strain was shown to inhibit the gliadin-induced increase of epithelial permeability in a dose-dependent manner *in vitro*. This strain influenced the zonulin expression pattern and inhibited membrane ruffle formation induced by gliadins in Caco-2 cells [54].

Commensal microbiota also interacts with the gut mucosa associated lymphoid tissue that contributes to diverse immune processes, such as secretory-IgA production, modulation of cytokine and chemokine release, and balanced Th1/Th2 responses and oral tolerance development to dietary antigens and commensal microbes (Fig. (**1**)) [55]. Specific *Bifidobacterium* strains have been acknowledged for their anti-inflammatory and regulatory properties by inducing IL-10 production in peripheral blood and lymphoid tissue cells [56, 57], in contrast to the predominant production of Th1-type cytokines induced by pathogenic microorganisms. In recent years, commensal bacteria, such as *B. infantis* 35624, have been shown to induce regulatory T cells, which contribute to protecting the host against aberrant activation of the innate immune system in response to pathogens [58]. Furthermore, transfer of probiotic-treated dendritic cells to mice has been shown to protect against 2, 4, 6-trinitrobenzenesulfonic acid-induced colitis. In this study, probiotics were shown to stimulate dendritic-cell regulatory functions associated with reduction of inflammatory scores and colonic expression of pro-inflammatory genes, and induction of CD4+ CD25+ regulatory cells in an IL-10-independent fashion [59]. On this basis, the use of certain strains as probiotics to treat or prevent inflammatory conditions, like inflammatory bowel diseases, has been proposed [60]. In addition, genetically modified bacterial strains have been constructed for mucosal delivery of bioactive proteins (e.g. IL-10, intestinal trefoil factor or superoxide dismutase) by oral administration. In particular, the administration of a *Lactococcus lactis* strain secreting recombinant IL-10 to genetically deficient IL-10 mice could counteract the development of chronic enterocolitis caused by an unregulated Th1 response to endogenous gut bacteria [61]. In a pilot study of 10

Crohn's disease patients, the oral intake of this strain also afforded certain benefits and had no toxic effects [62]. In recent studies, *Bifidobacterium* strains with immunoregulatory properties have been shown to suppress *in vitro* the pro-inflammatory cytokine pattern induced by the altered colonic microbiota of CD patients and to increase IL-10 production (Fig. (**1**)) [63]. In the light of this new evidence, probiotics have been proposed as novel dietary supplements that may help in the treatment and prevention of CD by regulating gut permeability and immune functions, without causing side-effects [50]. Preclinical and clinical *in vivo* studies have yet to be carried out to verify such a hypothesis.

CONCLUSIONS

The latest advances in understanding the pathogenic mechanisms underlying CD have contributed to discovering new molecular therapeutic targets of relevance to the future management of this disease. Although promising results can be anticipated on the strength of those reported for other chronic inflammatory disorders, the efficacy and safety of most therapeutic drugs have yet to be proven in CD patients by means of clinical trials. The fact that this pathology manifests clinically in a wide variety of ways would suggest that multiple genes, epigenetic factors and environmental elements are involved in its development. Furthermore, probably not all the patients are equally sensitive to the same gliadin fragments, which would make it particularly difficult to develop one single treatment for this disorder.

ACKNOWLEDGEMENTS

This work was supported by grants AGL2007-66126-C03-01/ALI and Consolider Fun-*C*-Food CSD2007-00063 from the Spanish Ministry of Science and Innovation. The author does not have any conflict of interest.

ABBREVIATIONS

CD = Celiac Disease

HLA = Human leukocyte antigen

IFN-γ = Interferon gamma

IL = Interleukin

tTG = Tissue transglutaminase

TNF-α = Tumor necrosis factor alpha

REFERENCES

[1] Green, P.H.; Jabri, B. Celiac disease. *Annu. Rev. Med.,* **2006**, *57*, 207-21.

[2] Fasano, A.; Catassi, C. Coeliac disease in children. *Best Pract. Res. Clin. Gastroenterol*., **2005**, *19*, 467-78.

[3] Black, K.E.; Murray, J.A.; David, C.S. HLA-DQ determines the response to exogenous wheat proteins: a model of gluten sensitivity in transgenic knockout mice. *J. Immunol.,* **2002**, *169*, 5595-600.

[4] Kagnoff, M.F. Overview and pathogenesis of celiac disease. *Gastroenterology,* **2005**, *128*, S10-8.

[5] Stenberg, P.; Roth, E.B.; Sjöberg, K. Transglutaminase and the pathogenesis of coeliac disease. *Eur. J. Intern. Med*., **2008**,*19*, 83-91.

[6] Gianfrani, C.; Auricchio, S.; Troncone R. Adaptive and innate immune responses in celiac disease. *Immunol. Lett*., **2005**, *99*, 141-5.

[7] Di Sabatino, A.; Ciccocioppo, R.; Cupelli, F.; Cinque, B.; Millimaggi,. D; Clarkson, M.M.; Paulli, M.; Cifone, M.G.; Corazza, G.R. Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease. *Gut,* **2006,** *55*, 469-77.

[8] Fasano, A.; Shea-Donohue, T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nat. Clin. Pract. Gastroenterol. Hepatol*., **2005**, *2*, 416-22.

[9] Tjellström, B.; Stenhammar, L.; Högberg, L.; Fälth-Magnusson, K.; Magnusson, K.E.; Midtvedt, T.; Sundqvist, T.; Norin, E. Gut microflora associated characteristics in children with celiac disease. *Am. J. Gastroenterol*., **2005,** *100,* 2784-8.

[10] Nadal, I.; Donat, E.; Ribes-Koninckx, C.; Calabuig, M.; Sanz, Y. Imbalance in the composition of the duodenal microbiota of children with coeliac disease. *J. Med. Microbiol*., **2007**, *56*, 1669-74.

[11] Collado, M.C.; Calabuig, M.; Sanz, Y. Differences between the fecal microbiota of coeliac infants and healthy controls. *Curr. Issues Intest. Microbiol*., **2007,** *8*, 9-14.

[12] Ivarsson, A.; Hernell, O.; Nyström, L.; Persson, L.A. Children born in the summer have increased risk for coeliac disease. *J. Epidemiol. Commun. Health,* **2003**, *57*, 36-9.

[13] Kondrashova, A.; Mustalahti, K.; Kaukinen, K.; Viskari, H.; Volodicheva, V.; Haapala, A.M.; Clonen, J.; Knip, M.; Mäki, M.; Hyöty, H. Epivir Study Group. Lower economic status and inferior hygienic environment may protect against celiac disease. *Ann. Med*., **2008**, *40*, 223-31.

[14] Rewers, M. Epidemiology of coeliac disease: what are the prevalence, incidence, and progression of coeliac disease? *Gastroenterology,* **2005**, *128*, S47-51.

[15] Shan, L.; Marti, T.; Sollid, L.M.; Gray, G.M.; Khosla, C. Comparative biochemical analysis of three bacterial prolyl endopeptidases: implications for coeliac sprue. *Biochem. J.,* **2004**, *383*, 311.

[16] Sanz, Y. In *Industrial Enzymes: Structure, Function and Applications*, Polaina, J.; McCabe, A., Eds. Springer: The Netherlands, **2007**; pp 243-260.

[17] Garcia-Horsman, J.A.; Venäläinen, J.I.; Lohi, O.; Auriola, I.S.; Korponay-Szabo, I.R.; Kaukinen, K.; Mäki, M.; Männistö, P.T. Deficient activity of mammalian prolyl oligopeptidase on the immunoactive peptide digestion in coeliac disease. *Scand. J. Gastroenterol*., **2007**, *42*, 562-71.

[18] Stepniak, D.; Spaenij-Dekking, L.; Mitea, C.; Moester, M.; de Ru, A.; Baak-Pablo, R.; van Veelen, P.; Edens, L.; Koning, F. Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. *Am. J. Physiol. Gastrointest. Liver Physiol*., **2006**, *291*, G621-9.

[19] Pyle, G.G.; Paaso, B.; Anderson, B.E.; Allen, D.D.; Marti, T.; Li, Q.; Siegel, M.; Khosla, C.; Gray, G.M. Effect of pretreatment of food gluten with prolyl endopeptidase on gluten-induced malabsorption in celiac sprue. *Clin. Gastroenterol. Hepatol*. **2005**, *3*, 687-94.

[20] Mitea, C.; Havenaar, R.; Drijfhout, J.W.; Edens, L.; Dekking, L.; Koning, F. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. *Gut*, **2008**, *57*, 25-32.

[21] Gass, J.; Bethune, M.T.; Siegel, M.; Spencer, A.; Khosla, C. Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. *Gastroenterology,* **2007,** *133*, 472-80.

[22] Savijoki, K.; Ingmer, H.; Varmanen, P. Proteolytic systems of lactic acid bacteria. *Appl. Microbiol. Biotechnol*., **2006**, *71*, 394-406.

[23] Di Cagno, R.; Rizzello, C.G.; De Angelis, M.; Cassone, A.; Giuliani, G.; Benedusi, A.; Limitone, A.; Surico, R.F.; Gobbetti, M. Use of selected sourdough strains of *Lactobacillus* for removing gluten and enhancing the nutritional properties of gluten-free bread. *J. Food. Prot*., **2008,** *71*, 1491-5.

[24] Molberg, O.; Mcadam, S.N.; Körner, R.; Quarsten, H.; Kristiansen, C.; Madsen, L.; Fugger, L.; Scott, H.; Norén, O.; Roepstorff, P.; Lundin, K.E.; Sjöström, H.; Sollid, L.M. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat. Med*., **1998**, *4*, 713-7.

[25] Zanoni, G.; Navone, R.; Lunardi, C.; Tridente, G.; Bason, C.; Sivori, S.; Beri, R.; Dolcino, M.; Valletta, E.; Corrocher, R.; Puccetti, A. In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. *PLoS Med.,* **2006**, *3*, e358.

[26] Myrsky, E.; Kaukinen, K.; Syrjänen, M.; Korponay-Szabó, I.R.; Mäki, M.; Lindfors, K. Coeliac disease-specific autoantibodies targeted against transglutaminase 2 disturb angiogenesis. *Clin. Exp. Immunol*., **2008**, *152*, 111-9.

[27] Siegel, M.; Xia, J.; Khosla, C. Structure-based design of alpha-amido aldehyde containing gluten peptide analogues as modulators of HLA-DQ2 and transglutaminase 2. *Bioorg. Med. Chem*., **2007,** *15*, 6253-61.

[28] Maiuri, L.; Ciacci, C.; Ricciardelli, I.; Vacca, L.; Raia, V.; Rispo, A.; Griffin, M.; Issekutz, T.; Quaratino, S.; Londei, M. Unexpected role of surface transglutaminase type II in celiac disease. *Gastroenterology,* **2005**, *129,* 1400-13.

[29] Choi, K.; Siegel, M.; Piper, J.L.; Yuan, L.; Cho, E.; Strnad, P.; Omary, B.; Rich, K.M.; Khosla, C. Chemistry and biology of dihydroisoxazole derivatives: selective inhibitors of human transglutaminase 2. *Chem. Biol*., **2005**, *12*, 469-75.

[30] Telci, G.; Griffin, M. Tissue transglutaminase (TG2)-a wound response enzyme. *Front. Biosci.,* **2006**, *11*, 867-82.

[31] Xia, J.; Bergseng, E.; Fleckenstein, B.; Siegel, M.; Kim, C.Y.; Khosla, C.; Sollid, L.M. Cyclic and dimeric gluten peptide analogues inhibiting DQ2-mediated antigen presentation in celiac disease. *Bioorg, Med, Chem*., **2007**, *15*, 6565-73.

[32] Silano, M.; Di Benedetto, R.; Trecca, A.; Arrabito, G.; Leonardi, F.; De Vincenzi, M. A decapeptide from durum wheat prevents celiac peripheral blood lymphocytes from activation by gliadin peptides. *Pediatr. Res*., **2007**, *61*, 67-71.

[33] Siegel, M.; Xia, J.; Khosla, C. Structure-based design of alpha-amido aldehyde containing gluten peptide analogues as modulators of HLA-DQ2 and transglutaminase 2. *Bioorg. Med. Chem*., **2007,** *15*, 6253-61.

[34] Van Deventer, S.J.; Elson, C.O.; Fedorak, R.N. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Disease Study Group. *Gastroenterology*, **1997**, *113*, 383-9.

[35] Salvati, V.M.; Mazzarella, G.; Gianfrani, C.; Levings, M.K.; Stefanile, R.; De Giulio, B.; Iaquinto, G.; Giardullo, N.; Auricchio, S.; Roncarolo, M.G.; Troncone, R. Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in *ex vivo* cultured coeliac intestinal mucosa. *Gut*, **2005**, *54*, 46-53.

[36] Mulder, C.J.; Wahab, P.J.; Meijer, J.W.; Metselaar, E. A pilot study of recombinant human interleukin-10 in adults with refractory coeliac disease. *Eur. J. Gastroenterol. Hepatol*., **2001**, *13*, 1183-8.

[37] Korzenik, J.R.; Podolsky, D.K. Evolving knowledge and therapy of inflammatory bowel disease. *Nat. Rev. Drug. Discov*., **2006**, *5,* 197-209.

[38] Peyrin-Biroulet, L.; Desreumaux, P.; Sandborn, W.J.; Colombel, J.F. Crohn's disease: beyond antagonists of tumour necrosis factor. *Lancet,* **2008**, *372*, 67-81.

[39] Di Sabatino, A.; Pickard, K.M.; Gordon, J.N.; Salvati, V.; Mazzarella, G.; Beattie, R.M.; Vossenkaemper, A.; Rovedatti, L.; Leakey, N.A.; Croft, N.M.; Troncone, R.; Corazza, G.R.; Stagg, A.J.; Monteleone, G.; MacDonald, T.T. Evidence for the role of interferon-alfa production by dendritic cells in the Th1 response in celiac disease. *Gastroenterology*, **2007**, *133*, 1175-87.

[40] Baslund, B.; Tvede, N.; Danneskiold-Samsoe, B.; Larsson, P.; Panayi, G.; Petersen, J.; Petersen, L.J.; Beurskens, F.J.; Schuurman, J.; van de Winkel, J.G.; Parren, P.W.; Gracie, J.A.; Jongbloed, S.; Liew, F.Y.; McInnes, I.B. Targeting interleukin-15 in patients with rheumatoid arthritis: a proof-of-concept study. *Arthritis Rheum*., **2005,** *52*, 2686-92.

[41] Ferrari-Lacraz, S.; Zanelli, E.; Neuberg, M.; Donskoy, E.; Kim, Y.S.; Zheng, X.X.; Hancock, W.W.; Maslinski, W.; Li, X.C.; Strom, T.B.; Moll, T. Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. *J. Immunol*., **2004,** *173*, 5818-26.

[42] Targan, S.R,; Feagan, B.G.; Fedorak, R.N.; Lashner, B.A.; Panaccione, R.; Present, D.H.; Spehlmann, M.E.; Rutgeerts, P.J.; Tulassay, Z.; Volfova, M.; Wolf, D.C.; Hernandez, C.; Bornstein, J.; Sandborn, W.J. International Efficacy of Natalizumab in Crohn's Disease Response and Remission (ENCORE) Trial Group. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE. Trial. *Gastroenterology,* **2007,** *132,* 1672-83.

[43] Rose, J.W.; Foley, J.; Carlson, N. Monoclonal antibody treatments for multiple sclerosis. *Curr. Neurol. Neurosci. Rep*., **2008**, *8*, 419-26.

[44] Feagan, B.G.; Greenberg, G.R.; Wild, G.; Fedorak, R.N.; Paré, P.; McDonald, J.W.; Dubé, R.; Cohen, A.; Steinhart, A.H.; Landau, S.; Aguzzi, R.A.; Fox, I.H.; Vandervoort, M.K. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. *N. Engl J Med*., **2005,** *352*, 2499-507.

[45] Bekker, P.; Schreiber, S.; Keshav, S. PROTECT-1, prospective randomized oral therapy evaluation of CCX282-B (TRAFICET-EN) in Crohn's disease. *Gut*, **2007**, *56* (Suppl), A23.

[46] Schulzke, J.D.; Bentzel, C.J.; Schulzke, I.; Riecken, E.O.; Fromm, M. Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. *Pediatr. Res*., **1998**, *43*, 435-41.

[47] Drago, S.; El Asmar, R.; Di Pierro, M.; Grazia Clemente, M.; Tripathi, A.; Sapone, A.; Tacar, M.; Iacono, G.; Carroccio, A.; D'Agate, C.; Not, T; Zampini, L.; Catáis, C.; Fasano, A. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand. J. Gastroenterol*., **2006,** *41*, 408-19

[48] Lammers, K.M.; Lu, R.; Brownley, J.; Lu, B.; Gerard, C.; Thoma, K.; Rallabhandi, P.; Shea-Donohue, T.; Tamiz, A.; Alkan, S.; Netzel-Arnett, S.; Antalis, T.; Vogel, S.N.; Fasano, A. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. *Gastroenterology*, **2008**, *135*, 194-204.e3.

[49] Paterson, B.M.; Lammers, K.M.; Arrieta, M.C.; Fasano, A.; Meddings, J.B. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. *Aliment Pharmacol. Ther*., **2007**, *26*, 757-66.

[50] Sanz, Y.; Sánchez, E.; De Palma, G.; Medina, M; Marcos, A.; Nova, E. In *Child Nutrition & Physiology,* Linda, T.O.; Ewente, M R., Eds.; Nova Science Publishers Inc: New York, **2008**.

[51] Li, Q.; Zhang, Q.; Wang, M.; Zhao, S.; Ma, J.; Luo, N.; Li, N.; Li, Y.; Xu, G.; Li, J. Interferon-gamma and tumor necrosis factor-alpha disrupt epithelial barrier function by altering lipid composition in membrane microdomains of tight junction. *Clin. Immunol*., **2008**, *126*, 67-80.

[52] Zeng, J.; Li, Y.Q.; Zuo, X.L.; Zhen, Y.B.; Yang, J.; Liu, C.H. Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhea-predominant irritable bowel syndrome. *Aliment. Pharmacol. Ther*., **2008**, *28*, 994-1002.

[53] Seth, A.; Yan, F.; Polk, D.B.; Rao, R.K. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am. J. Physiol. Gastrointest. Liver Physiol*., **2008,** *294*, G1060-9.

[54] Lindfors, K.; Blomqvist, T.; Juuti-Uusitalo, K.; Stenman, S.; Venäläinen, J.; Mäki, M.; Kaukinen, K. Live probiotic *Bifidobacterium lactis* bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. *Clin. Exp. Immunol*., **2008**, *152*, 552-8.

[55] Sanz, Y.; Nadal, I.; Sánchez, E. Probiotics as drugs against human gastrointestinal infections. *Recent Patents Anti-Infect. Drug Discov*., **2007**, *2,* 148-56.

[56] Medina, M.; Izquierdo, E.; Ennahar, S.; Sanz, Y. Differential immunomodulatory properties of *Bifidobacterium longum* strains: relevance to probiotic selection and clinical applications. *Clin. Exp. Immunol.,* **2007**, *150*, 531-8.

[57] Baba, N.; Samson, S.; Bourdet-Sicard, R.; Rubio, M.; Sarfati, M. Commensal bacteria trigger a full dendritic cell maturation program that promotes the expansion of non-Tr1 suppressor T cells. *J. Leukoc. Biol.,* **2008**, *84*, 468-76.

[58] O'Mahony, C.; Scully, P; O'Mahony, D.; Murphy, S.; O'Brien, F.; Lyons, A.; Sherlock, G.; MacSharry, J.; Kiely, B.; Shanahan, F.; O'Mahony, L. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation. *PLoS Pathog*., **2008**, 4, 8:e1000112.

[59] Foligne, B.; Zoumpopoulou, G.; Dewulf, J.; Ben Younes, A.; Chareyre, F.; Sirard, J.C.; Pot, B.; Grangette, C. A key role of dendritic cells in probiotic functionality. *PLoS ONE*, **2007,** *2*, e313.

[60] Guarner, F.; Bourdet-Sicard, R.; Brandtzaeg, P.; Gill, H.S.; McGuirk, P.; van Eden, W.; Versalovic, J.; Weinstock, J.V.; Rook, G.A. Mechanisms of disease: the hygiene hypothesis revisited. *Nat. Clin. Pract. Gastroenterol. Hepatol*., **2006**, *3*, 275-284.

[61] Steidler, L.; Hans, W.; Schotte, L.; Neirynck, S.; Obermeier, F.; Falk, W.; Fiers, W.; Remaut, E. Treatment of murine colitis by

Lactococcus lactis secreting interleukin-10. *Science*, **2000**, *289*, 1352-5.

[62] Braat, H.; Rottiers, P.; Hommes, D.W.; Huyghebaert, N.; Remaut, E.; Remon, J.P.; van Deventer, S.J.; Neirynck, S.; Peppelenbosch, M.P.; Steidler, L. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin. Gastroenterol. Hepatol*., **2006**, *4*, 754-9.

[63] Medina, M.; De Palma, G.; Ribes-Koninckx, C.; Calabuig, M.; Sanz, Y. *Bifidobacterium* strains suppress *in vitro* the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. *J. Inflamm. (Lond.),* **2008**, *5*, 19.

Received: 11 September, 2008 Revised: 15 December, 2008 Accepted: 09 January, 2009