Polymeric Binders Suppress Gliadin-Induced Toxicity in the Intestinal Epithelium

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Background & Aims: Celiac disease is a prevalent immune disorder caused by the ingestion of gliadin-containing grains. We investigated the ability of a polymeric binder to reverse the toxic effects induced by gliadin in human intestinal cells and gliadin-sensitive HCD4-DQ8 mice. Methods: Gliadin was neutralized by complexation to a linear copolymer of hydroxyethylmethacrylate (HEMA) and sodium 4-styrene sulfonate (SS). The ability of the polymeric binder to abrogate the damaging effect of gliadin on cell-cell contact was investigated in IEC-6, Caco-2/15, and primary cultured differentiated enterocytes. The efficacy of the polymeric binder in preventing gliadin-induced intestinal barrier dysfunction was assessed using gliadin-sensitive HLA-HCD4/DQ8 transgenic mice. Results: Poly(hydroxyethylmethacrylate-co-styrene sulfonate) [P(HEMA-co-SS)] complexed with gliadin in a relatively specific fashion. Intestinal cells exposed to gliadin underwent profound alterations in morphology and cell-cell contacts. These changes were averted by complexing the gliadin with P(HEMA-co-SS). More importantly, the P(HEMA-co-SS) hindered the digestion of gliadin by gastrointestinal enzymes, thus minimizing the formation of immunogenic peptides. Coadministration of P(HEMA-co-SS) with gliadin to HLA-HCD4/DQ8 mice attenuated gliadin-induced changes in the intestinal barrier and reduced intraepithelial lymphocyte and macrophage cell counts. Conclusions: Polymeric binders can prevent in vitro gliadin-induced epithelial toxicity and intestinal barrier dysfunction in HCD4/DQ8 mice. They have a potential role in the treatment of patients with gluten-induced disorders.

Celiac disease (CD) is an immune enteropathy triggered by the ingestion of gluten-containing grains (wheat, barley, rye) in susceptible individuals. Screening studies have demonstrated that CD is one of the most frequent genetically based diseases, occurring in 1 out of 200–300 people worldwide and up to 1% of the white ethnic population. Moreover, milder forms of gluten intolerance have been recognized as a possible contributor to irritable bowel syndrome even in the absence of full-blown CD. The development of the disease is closely associated to genes that code for human leukocyte antigens (HLA) DQ2 and DQ8, molecules of the major histocompatibility complex and to other non-HLA genes. Recently, specific peptide fragments of α-gliadin (a gluten prolamine) were identified as possible triggers for the response to gluten in CD patients. Three of the most immunogenic epitopes are contained in a 33-amino acid peptide (57–89) that is exceptionally resistant to enzymatic processing because of its high content in proline and glutamine. These 3 epitopes induce T-cell proliferation via the interaction antigen-presenting cells and HLA-DQ2/DQ8. The toxicity of these motifs was found to be even greater after deamination of specific glutamine residues by tissue transglutaminases. On the other hand, the nonimmunodominant 31–43 peptide triggers an innate response via interleukin (IL)-15. To date, no pharmacologic treatment is available to gluten-intolerant patients. This can lead to poorly controlled disease patients because poor adherence to a gluten-free diet, whether voluntary or inadvertent, is common. Various therapeutic avenues are currently being explored to tackle this pathology. These include administration of (1) bacterial endopeptidases or probiotics that can completely digest the toxic gluten-derived peptides, (2) inhibitors of the zonulin pathway, (3) inhibitors of tissue transglutaminases, (4) peptides that block the binding of immunogenic gliadin fragments to DQ2 and DQ8, (5) recombinant IL-10 to suppress gliadin-dependent T-cell activation, and (6) antibodies neutralizing IL-15 or interferon (IFN)-γ. Other nonmedical approaches consist in generating gluten-free dietary products, using fermentation as a means to degrading gluten and its

Abbreviations used in this paper: CD, celiac disease; HEMA, hydroxyethylmethacrylate; HLA, human leukocyte antigen; HRP, horseradish peroxidase; IEL, intraepithelial lymphocyte; PCDE, primary cultures of human differentiated enterocytes; PEG, poly(ethylene glycol); P(HEMA-co-SS), poly(hydroxyethylmethacrylate-co-styrene sulfonate); PSS, polystyrene sulfonate; SS, styrene sulfonate; ZO-1, zonula occludens-1.

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0016-5085/09/$36.00
derivatives contained in cereals or chemically modifying gliadin to block the T-cell response.

In this work, a yet unexplored strategy based on synthetic polymeric binders that complex and neutralize gliadin in situ was investigated. Polymeric binders are high molecular weight polymers that are used to reduce the intestinal absorption of endogenous or exogenous molecules. They act in the gastrointestinal tract without being absorbed into the bloodstream, thereby minimizing the potential toxicity and adverse effects.

Materials and Methods

Materials

α-Gliadin (from whole wheat var. Triticum vulgare) was supplied by Prof Popineau (Institut National de la Recherche Agronomique, Nantes, France). Bovine serum albumin (BSA) was purchased from Serological Proteins (Kankakee, IL). Poly(hydroxyethylmethacrylate-co-styrene sodium sulfate) (P(HEMA-co-SS) (45:55 ± 2 mol%, 40 kilodaltons) was synthesized as described in the supplementary materials section (see supplementary materials online at www.gastrojournal.org).

The Zonula occludens-1 (ZO-1) peptide 31–43mer (LGQQQPFPPQQPY) was purchased from Sigma–Genosys (The Woodlands, TX). The routine subculture was carried out at confluence. Primary cultures of human differentiated enterocytes (PCDE) were prepared from fetal human small intestines ranging from 16 to 20 weeks of age using a nonenzymatic procedure (Matrisperse Cell Recovery solution; BD Bioscience) and cultured as described by Perreault and Beaulieu. The protocol was approved by the Institutional Human Research Review Committee of University of Sherbrooke for the use of human material. All cells were cultured in a humidified atmosphere containing 5% CO2 at 37°C. The binders used in all cell assays were polystyrene sulfonate (PSS) (50 kilodaltons) and P(HEMA-co-SS) dispersed in culture medium.

Immunofluorescence Microscopy

IEC-6 cells were seeded and grown on Lab-Tek chamber slides (Nalgen Nunc International, Rochester, NY) for 2 days prior to the experiments. For F-actin staining, cells were incubated for 30 minutes in various media, washed twice with phosphate-buffered saline (PBS), fixed in paraformaldehyde 3% for 15 minutes at room temperature, and permeabilized with 0.1% Triton X-100 for 10 minutes. Thereafter, cells were blocked in PBS containing 2% BSA for 20 minutes at room temperature, after which they were incubated for 30 minutes with phalloidin-FITC and washed 3 times with PBS. Caco-2/15 cells were grown for 8 days after reaching confluence and then processed as previously described to obtain longitudinal sections that were immunostained for ZO-1.

Cell Viability Assay

Viability of PCDE treated or not with whole gliadin in the presence or absence of P(HEMA-co-SS) was monitored by the trypan blue exclusion test.
**In Vivo Efficacy Experiments**

All experiments were approved by the McMaster or Montreal University Animal Care Committees, in accordance with the Canadian Council on Animal Care guidelines. Transgenic male mice (~26 g), 6 to 8 weeks old, and expressing HLA-HCD4/DQ8 (HLA-DQA1*0301; HLA-DQb1*0302) genes in the absence of endogenous mouse class II genes were used. The animals were sensitized to gliadin as previously described. Briefly, mice were injected intraperitoneally with 500 μg of gliadin dissolved in 0.02 mol/L acetic acid plus 50 μL of complete Freund’s adjuvant. Nonsensitized mice (controls) received complete Freund’s adjuvant intraperitoneally only. Starting 1 week later, gliadin challenge was performed 3 times a week by intragastric gavage, for 3 weeks, using 2 mg of whole gliadin dissolved in 0.02 mol/L acetic acid. Four groups of mice were used: (1) nonsensitized mice, (2) nonsensitized mice gavaged with P(HEMA-co-SS) (6 mg in water), (3) gliadin-sensitized mice gavaged with gliadin (2 mg), (4) gliadin-sensitized mice gavaged with gliadin (2 mg) and P(HEMA-co-SS) (6 mg). P(HEMA-co-SS) was administered 5 minutes prior to the gliadin gavage. Mice were killed 24 hours after the last gavage, and cross sections from proximal small intestine were preserved in 10% formalin then processed and stained with H&E. Specimens were examined under light microscopy for epithelial damage.

**Intestinal permeability.** Two sections of the jejunum and the ileum from each mouse were used for epithelial ion and macromolecule transport measurements. Briefly, 5-cm sections of tissues were removed and divided into 2 segments. Each segment was opened along the mesenteric border, rinsed, and mounted in an Ussing chamber (exposed surface area, 0.6 cm²). Tissues were bathed in oxygenated Krebs buffer containing 10 mmol/L glucose (serosal side) or 10 mmol/L mannitol (luminal side) at 37°C. The net active transport across the epithelium was measured via a short-circuit current injected through the tissue under voltage clamp conditions. After a 15-minute equilibration period, baseline short-circuit current and conductance were recorded. Horseradish peroxidase (HRP) was used as a model protein to probe macromolecular permeability. HRP was added (10⁻⁵ mol/L) to the luminal buffer once equilibrium was reached. Serosal samples (500 μL) were taken at 30-minute intervals for 2 hours and replaced with fresh buffer to maintain constant volume. The enzymatic activity of HRP was measured using a modified Worthington method as previously described.

**Immunohistochemistry.** Immunostaining for CD3⁺ cells was performed on paraffin sections as described elsewhere. A mean value was obtained and expressed as the number of positive cells per 5 villi. Intraepithelial lymphocytes (IELs)/20 enterocytes in 5 randomly chosen villous tips were counted according to the method of Biagi et al and expressed as IEL/100 enterocytes. Immunostaining for macrophages was performed on paraffin sections as described previously. Positive cells for F4/80 staining were counted in the lamina propria in 5 villi randomly chosen using 2 different sections per mouse. A mean value was obtained and expressed as the number of macrophage-positive cells per 5 villi.

**In Vivo Toxicity Experiments**

Wild-type male Balb/c mice (~18 g) were used to assess the safety of P(HEMA-co-SS). Mice (n = 7/group) were given P(HEMA-co-SS) or poly(ethylene glycol) (PEG; 35 kilodaltons) dissolved in the drinking water (3 mg/mL). Control mice received water only. Mice were weighed daily for 22 days, and the daily drinking water consumption was estimated (~5 mL/day). The mice were then killed, and the jejunum, stomach, and liver were harvested and analyzed histologically for signs of toxicity. H&E-stained slides of the organs were examined under optical microscopy. Blood samples were analyzed for biochemical and cellular parameters by the Quebec Transgenic Research Network.

**Statistical Analysis**

Mean and median of each group were compared and homoscedasticity was verified using F test to ensure the normal distribution. Next, group values were compared using a parametric analysis of variance (ANOVA) test, followed by Tukey test to determine the significance of all paired combinations.

**Results**

P(HEMA-co-SS) Binds Gliadin

α-Gliadin is a poorly water soluble protein with an isoelectric point of ~6. Therefore, it is positively charged as occurs in the stomach, at a pH of 1.2, and almost neutral and highly hydrophobic at the intestinal pH (5.5–7). To maximize affinity for gliadin at both low and high pH values, styrene sulfonate (SS), a monomer capable of forming both electrostatic and hydrophobic interactions with the protein through its sulfonated aromatic ring, was incorporated in the chelating polymer. SS units were copolymerized with hydroxyethylmethacrylate (HEMA). The latter hydrophilic monomer was introduced to favor hydrogen bonding, but mainly to ensure the biocompatibility of the binder. As shown in Figure 1A, the copolymer successfully complexed with gliadin at pH 6.8. At that pH, the charge of α-gliadin is approximatively neutral; the interactions with the copolymer are either hydrophobic in nature or because of hydrogen bonds. The influence of polymer composition on complex formation was investigated by SDS-PAGE at gastric and intestinal pHs. Under acidic conditions, P(HEMA-co-SS) exhibited a strong binding to gliadin (>75%), irrespective of the SS content. Conversely, at pH 6.8, the affinity of gliadin for the polymer strongly de-
pend on the SS content. The binding increased with the SS amount and levelled off at approximately 55 mol% (see supplementary Figure 1 online at www.gastrojournal.org). The specificity of the polymer-gliadin interaction was examined at pH 6.8 in the presence of several other proteins commonly found in food such as BSA, ovalbumin, and lysozyme. A cocktail of these different proteins was incubated with P(HEMA-co-SS) in the presence of gliadin and analyzed by SDS-PAGE. As can be seen from Figure 1B and C, the copolymer interacted preferentially with the highly hydrophobic α-gliadin, compared with most other hydrophilic food proteins/peptides. Indeed, the observed binding efficiency was ~4-times for gliadin compared with albumin at the same protein concentration.

**P(HEMA-co-SS) Does Not Perturb IEC-6 Cytoskeleton and Morphology**

The effect of various polymers on the integrity of the cytoskeleton of IEC-6 intestinal epithelial cells was first monitored by staining the F-actin with phalloidin-FITC (Figure 2). Two binders, namely P(HEMA-co-SS) and the SS homopolymer (PSS), were compared with PEG, a well-known innocuous polymer PEG. Cells incubated with either PEG (Figure 2, panel i, arrow) or P(HEMA-co-SS) (Figure 2, panel ii, arrow) did not experience any changes in morphology, and the staining of F-actin revealed intact cell-cell contacts. Moreover, neither polymer affected cell viability. In contrast, PSS induced a rapid loss of cell-cell contacts as shown in Figure 2 (panel iii, arrow). In vitro, PSS was shown to possess a greater affinity for phospholipid membranes compared with P(HEMA-co-SS) (data not shown). Thus, PSS may influence cellular membrane functioning or cell signalling processing leading to cell damages. Therefore, further investigations were pursued only with P(HEMA-co-SS).

**P(HEMA-co-SS) Abolishes the Deleterious Effect of Gliadin on the Cell Cytoskeleton and Junctional Protein, ZO-1**

Next, we investigated whether P(HEMA-co-SS) could prevent the direct effects of gliadin on intestinal epithelial cells. Gliadin has been reported to induce cytoskeleton reorganization and ultrastructural damages in cultured intestinal epithelial cells. The incubation of IEC-6 cells with gliadin decreased the number of intracellular actin filaments (Figure 3A). In addition, the cells treated with gliadin were loosely connected and irregularly shaped (Figure 3A, panel ii, arrow). Comparatively, no significant changes were observed when cells were exposed to BSA at a similar concentration (Figure 3A, panel i, arrow). The effect of gliadin on the actin cytoskeleton was partially or completely prevented by the coaddition of P(HEMA-co-SS) at a gliadin/polymer weight ratio of 1:1 (Figure 3A, panel iii, arrow) or 1:2 (Figure 3A, panel iv, arrow), respectively.

To analyze the impact of gliadin on tight junctions, the human colon cancer cell line Caco-2/15 was used. It provides a unique and well-characterized model for the study of intestinal epithelial differentiation. On longitudinal sections, immunostaining of differentiated Caco-
2/15 cells (8 days postconfluence) confirmed that the immunoreactivity of ZO-1 was restricted to the apex of the lateral cell membrane (Figure 3B, panel i, arrows). When these cells were treated with gliadin, ZO-1 immunolocalization in the apex was partially or completely lost (Figure 3B, panel ii, arrows), as recently reported. However, ZO-1 immunoreactivity was progressively restored upon the coaddition of increasing amounts of P(HEMA-co-SS) (Figure 3B, panels iii and iv, arrows).

**P(HEMA-co-SS) Abolishes the Cytotoxic Effect of Gliadin on PCDE**

The cytotoxicity of gliadin, alone or complexed with P(HEMA-co-SS), was evaluated on PCDE. After

![Figure 2](image1.png)

**Figure 2.** Effect of chelating polymer on intestinal epithelial cell cytoskeleton and morphology. IEC-6 cells were incubated for 30 minutes with 1 mg/mL of PEG (negative control) (i), P(HEMA-co-SS) (ii), and PSS (iii). White arrows indicate F-actin at cell-cell contacts in panels i and ii and disruption of cell-cell contact in panel iii.

![Figure 3](image2.png)

**Figure 3.** Effect of gliadin complexation with P(HEMA-co-SS) on intestinal epithelial cell cytoskeleton (A) and junctional protein ZO-1 (B). (A) IEC-6 cells were incubated for 30 minutes with 0.5 mg/mL of BSA (i), gliadin alone (ii), or in combination with P(HEMA-co-SS) at a gliadin/polymer weight ratio of 1:1 (iii) and 1:2 (iv). White arrows indicate cell-cell contacts in panels i, iii, and iv and a disruption of cell-cell contacts in panel ii. Data shown are representative of 3 different experiments. (B) Caco-2/15 cells were incubated for 30 minutes with 0.5 mg/mL BSA (i), gliadin alone (ii), or in combination with P(HEMA-co-SS) at a gliadin/polymer weight ratio of 1:1 (iii) and 1:2 (iv). White arrows in panels i, iii, and iv point to the ZO-1 protein. White arrows in panel ii indicate the lost of ZO-1 staining.
48-hour incubation with gliadin, disruptions occurred in the cell lawn, as evidenced by the presence of empty areas (see supplementary Figure 2 online at www.gastrojournal.org). The addition of P(HEMA-co-SS) at a gliadin/polymer weight ratio of 1:2 was able to revert this effect back to that of normal cells (ie, treated with BSA). Cell mortality was quantified using the trypan blue assay. As illustrated in Figure 4, treatment of PCDE with gliadin during 48 hours significantly increased cell mortality in comparison with BSA. The sensitivity of this cell model to gliadin can be explained by the absence of mesenchyme or by the Matrisperse treatment. Importantly, the cytotoxic effect of gliadin was abolished by P(HEMA-co-SS). Moreover, incubation with P(HEMA-co-SS) did not alter the viability of PCDE even after 48 hours.

**P(HEMA-co-SS) Hinders the Enzymatic Hydrolysis of Gliadin**

The ability of the polymeric binder to protect α-gliadin from enzymatic digestion was assessed by high-performance liquid chromatography analysis. When gliadin was incubated in presence of P(HEMA-co-SS), no native protein and less degradation products were detected (see supplementary Figure 3 online at www.gastrojournal.org). For example, compared with the uncomplexed gliadin (Figure 5), the area under the peak at the elution time of 9.5 minutes decreased by 50% and 80% in the presence of P(HEMA-co-SS) at 1:1 (ii) and 1:2 (iii) gliadin/polymer weight ratios. The data suggest a decrease in the formation of the immunomodulatory peptide as this peptide elutes at 9.5 minutes.

**P(HEMA-co-SS) Abolishes the Gliadin-Induced Effects on Barrier Function in HCD4/DQ8 Mice**

To test whether P(HEMA-co-SS) could reverse intestinal barrier abnormalities induced by gliadin in vivo, gluten sensitive HLA-DQ8/HCD4 transgenic mice were employed. As shown before, gliadin-sensitized HLA-DQ8/HCD4 mice gavaged with gliadin demonstrated significant increases in baseline ion transport (short-circuit current) (Figure 6A) and increased HRP flux (Figure 6B) compared with nonsensitized controls. The hypersecretory state and increased macromolecular transport observed in gluten-sensitive mice were normalized in animals treated with P(HEMA-co-SS) administered orally 5 minutes prior to gliadin. Additionally, administration of P(HEMA-co-SS) alone to mice did not alter barrier function. Finally, intestinal permeability measurements in the ileum was similar between the negative control animals and those receiving P(HEMA-co-SS) prior to gliadin gavage (see supplementary Figure 4 online at www.gastrojournal.org). The data suggest that the deleterious effects of gliadin did not occur farther down in the gastrointestinal tract in the presence of the binder.
Increased CD3$^+$ and F4/80 Counts in Gliadin-Sensitive HCD4/DQ8 Mice Are Attenuated by P(HEMA-co-SS)

The analysis of H&E-stained slides under optical microscopy did not reveal villous atrophy in any group (see supplementary Figure 5 online at www.gastrojournal.org). Nevertheless, gliadin induced increased CD3$^+$ and F4/80 cell counts in sensitized HCD4/DQ8 mice, as expected. Interestingly, treatment with the polymeric binder abrogated the cellular response to gliadin, bringing the CD3$^+$IEL and macrophage counts back to the control levels. Mice exposed to P(HEMA-co-SS) alone had CD3$^+$IEL (Figure 7A) and F4/80$^+$ cell counts (Figure 7B) comparable with the nonsensitized controls. During this experiment, mice were monitored on a weekly basis for signs of toxicity. No clinical adverse effects were detected, and no weight loss was recorded in any of the groups (see supplementary Figure 6 online at www.gastrojournal.org).

P(HEMA-co-SS) Is Safe In Vivo When Administered at High Doses

To assess further the safety of P(HEMA-co-SS), the polymer was administered to healthy Balb/c mice at a high dose of 15 mg/day (830 mg/kg/day) for 22 days. Control groups consisted of mice receiving water or PEG. Mice were monitored 6 times a week for signs of distress, such as hunching, immobility, lack of grooming, and weight loss. No clinical ill effect was recorded in either group. At the end of the experiment, all 3 groups exhibited a similar weight gain (Figure 8A). Moreover, there was no difference between their biochemical and cellular clinical parameters (see supplementary Table 1 online at www.gastrojournal.org). However, the histologic analysis of the jejunum (Figure 8B), liver, and stomach did not reveal any abnormalities (data not shown).

Discussion

In this paper, we report for the first time a sequestering polymer that may prove useful in the clinical management of gluten intolerance and CD, conditions for which there are no pharmacologic treatments. Polymeric binders are commonly used in clinical situations to sequester small endogenous molecules such as potassium, bile salts, and phosphates. When given orally, polymeric sequestrants are not absorbed and are usually well tolerated even upon chronic ingestion of large doses (up to 6–15 g/day). Recently, an SS polymer was reported to neutralize the Clostridium difficile toxin A, a common cause of severe nosocomial diarrhea.

Our results show that a copolymer of SS and HEMA can selectively complex gliadin and abolish the direct toxic effects of the protein on rat and human IECs (IEC-6, Caco-2/15, PCDE). The protective effect of P(HEMA-co-SS) on the cell-cell contacts and integrity of ZO-1, a membrane-associated protein involved in tight

Figure 6. P(HEMA-co-SS) abolishes the gliadin-induced barrier dysfunction in HCD4/DQ8 mice. Controls consisted of nonsensitized mice gavaged with or without P(HEMA-co-SS). Treatment groups consisted of gliadin-sensitized mice gavaged with whole gliadin with or without polymer (n = 6 or 7/group). Administration of P(HEMA-co-SS) significantly decreased baseline ion transport (A) and HRP flux (B) compared with untreated gliadin-sensitized mice. *P ≤ .02.
Figure 7. P(HEMA-co-SS) significantly decreased CD3+ (A) and F4/80+ cells (B) compared with untreated gliadin-sensitized mice. Immunohistochemistry and histogram show the number of CD3+ IEL and elongated F4/80+ cells in the lamina propria: (i) nonsensitized mouse gavaged with rice, (ii) nonsensitized mouse challenged with P(HEMA-co-SS), (iii) gliadin-sensitized mouse challenged with gliadin, and (iv) gliadin-sensitized mouse challenged with gliadin and P(HEMA-co-SS). *P ≤ .05.
junctions, is especially meaningful. Gliadin and its immunomodulatory fragments have indeed been shown to induce a MyD88-dependent zonulin release that causes opening of tight junctions in subjects with dysregulation of the zonulin system. This was found to enable the paracellular translocation of gliadin and its digested fragments and their subsequent interaction with macrophages within the intestinal submucosae. By sequestering gliadin, P(HEMA-co-SS) may prevent such events to occur.

Figure 8. (A) Weights of mice fed with water (circle), PEG (triangle), and P(HEMA-co-SS) (square) at an average polymer dose of 15 mg/day. Administration of P(HEMA-co-SS) did not change significantly the weight gain after 22 days (mean ± SD). (B) H&E-stained sections of jejunum in mice which received water (i), PEG (ii), and P(HEMA-co-SS) (iii).
The most dramatic property of the P(HEMA-co-SS) was its ability to slow down the enzymatic digestion of gliadin. In particular, P(HEMA-co-SS) brought about an 80% reduction of immunogenic peptide 31–43 containing peak that is involved in the innate response because of IL-15. It has recently been suggested that the intestinal barrier could be the initial target of gliadin peptides, initiating a cascade of events that, in a genetically predisposed host, leads to gluten-induced pathology. The effectiveness of P(HEMA-co-SS) was further tested in a murine model of gluten sensitivity. Its ability to block the gliadin-induced alteration of intestinal barrier parameters and the increase in CD3+IEL and F4/80+ lamina propria cell counts suggest that efficient gliadin sequestration can occur in vivo.

To date, a few experimental therapies have been tested with variable results. One of the simplest experimental therapeutic strategies consists in using probiotics and/or exogenous enzymes to cleave the immunomodulatory peptides and reduce their luminal concentration. For example, prolylendopeptidase and endoprotease EP-B2, used alone or in combination, were shown to alleviate gluten toxicity under simulated intestinal conditions. On wild-type animals, the EP-B2/gluten diet significantly decreased the formation of the 33mer peptide. A preliminary clinical study using prolylendopeptidase was performed by Khosla et al. The fact that some patients responded to a prolylendopeptidase digest that had apparently lost its immunogenicity when tested in vitro on gliadin-specific T-cell lines remains puzzling. A second clinical assay based on oral enzyme therapy has recently been published by Cornell et al. Histologic improvement was not major in the few tested patients, and the changes in antitissue transglutaminases antibodies were modest. Even if those results suggest some protection, they emphasize the need for complementary approaches.

The enzymatic and the chelating strategies share similarities such that they are not aimed at reinstating a normal diet but rather at improving the quality of life of poorly controlled CD patients by reducing the immunogenic peptide burden below a tolerated threshold. To ensure a greater control over the disease, sequestering polymers may be used in conjunction with other investigated drugs such as inhibitors of tissue transglutaminases, peptides blocking the binding of immunogenic gliadin fragments to DQ2 and DQ8 and inhibitors of the zonulin pathway.

In conclusion, P(HEMA-co-SS) supplementation has the potential to become a safe adjuvant therapy for the control of gluten-induced disorders. Future work will be aimed at investigating the effect of the binder on the immune response triggered by gliadin on human biopsy specimens from CD patients, as well as assessing its efficacy in the presence of gluten-containing cereals.

References


Received February 22, 2008. Accepted September 11, 2008.

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The authors disclose the following: Supported by the Canadian Celiac Association, Fonds Québécois de la Recherche sur la Nature et les Technologies, Canadian Institutes of Health Research (to N.R.), National Institutes of Health DK 071003 (to C.S.D.), CAG/CIHR New Investigator Establishment Award (to E.F.V.), and the Canada Research Chair program.