Minireview

In vitro models for gluten toxicity: relevance for celiac disease pathogenesis and development of novel treatment options

Katri Lindfors[1], Tiina Rauhavirta[1], Satumarja Stenman[1], Markku Mäki[1] and Katri Kaukinen[2,3]

[1]Pediatric Research Center, University of Tampere and Tampere University Hospital; [2]School of Medicine, University of Tampere, 33014 Tampere; [3]Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, 33521 Tampere, Finland
Corresponding author: Dr Katri Lindfors, Pediatric Research Center, School of Medicine, Finn-Medi 3, University of Tampere, Tampere 33014, Finland. Email: katri.lindfors@uta.fi

Abstract

In genetically predisposed individuals, dietary gluten in wheat, rye and barley triggers celiac disease, a systemic autoimmune disorder hallmarked by an extensive small-bowel mucosal immune response. The current conception of celiac disease pathogenesis is that it involves components of both innate and adaptive immunity whose activation typically leads to small-bowel villous atrophy with crypt hyperplasia. Currently, the only effective treatment for celiac disease is a strict lifelong gluten-free diet excluding all wheat-, rye- and barley-containing food products. During the diet, the clinical symptoms improve and the small-bowel mucosal damage recovers, while re-introduction of gluten into the diet leads to re-appearance of the symptoms and deterioration of the small-bowel mucosal architecture. In view of the restricted nature of the diet, alternative treatment is warranted. Improved understanding of the molecular basis of celiac disease has enabled researchers to suggest other therapeutic approaches. Although there is no animal model reproducing all features of celiac disease, the use of *in vitro* approaches including a variety of cell lines and the celiac patient small-bowel mucosal biopsy organ culture has generated knowledge about pathogenesis of celiac disease. In these culture systems, gluten induces different effects that can be quantified, thus also enabling studies concerning the efficacy of candidate therapeutic compounds for celiac disease. This review describes the intestinal epithelial cell models, celiac patient T-cell lines and clones, as well as the small-bowel mucosal organ culture methods widely used in studies of celiac disease, and summarizes the major findings obtained with these systems.

Keywords: celiac disease, gluten, epithelium, T-cells, organ culture

Experimental Biology and Medicine 2012; **237**: 119–125. DOI: 10.1258/ebm.2011.011294

Introduction

Dietary gluten in wheat, rye and barley is the etiological agent in celiac disease. In a subgroup of individuals carrying the human leukocyte antigen (HLA) DQ2 or DQ8 molecules, ingestion of gluten leads to a small-bowel mucosal immune response and development of celiac disease. The disorder often presents with small-bowel villous atrophy, crypt hyperplasia and immune activation, including the production of disease-specific antibodies against gluten-derived gliadin peptides and an endogenous protein transglutaminase 2 (TG2).[1] The current conception of celiac disease pathogenesis is that it involves components of both innate and adaptive immunity. Gluten contains a high amount of repetitive glutamine- and proline-rich sequences, making it highly resistant to proteolytic degradation by gastrointestinal enzymes.[2,3] This results in the persistence of relatively large gluten-derived gliadin peptides which predispose to activation of the small-bowel mucosal immune system. Some of the gliadin peptides (the so-called toxic peptides, for example p31–43) are able to induce harmful effects on the small-intestinal epithelium through interleukin (IL)-15, whose overexpression eventually leads to epithelial cell destruction.[4,5] Other gliadin peptides, the immunodominant ones (for example p57–68 and the 33-mer) in turn activate T-cell-mediated adaptive immunity in the mucosa. A key step in this process is the post-translational modification of immunogenic gliadin peptides by TG2. TG2 has the capacity to deamidate distinct glutamine residues in gliadin peptides to glutamic acid, which favors their interaction with the celiac-type HLA DQ2, thereby promoting the activation of T-cells.[3,6,7]

Similar to other autoimmune disorders, celiac disease is self-perpetuating if the specific exogenous trigger, gluten, is not removed. Its removal by a strict gluten-free diet

leads to recovery of the small-bowel mucosal lesion and disappearance of the clinical symptoms. Re-introduction of gluten into the diet, on the other hand, results in the re-appearance of clinical symptoms and deterioration of the small-bowel mucosal architecture. Today, the only effective treatment for celiac disease is a lifelong gluten-free diet excluding all wheat-, rye- and barley-containing food products. As the diet is restricted and often burdensome, novel treatment options would be highly welcomed by celiac patients. During recent years, several lines of research have in fact aimed to develop alternative therapies. Approaches include blocking of innate and adaptive immune components as well as the function of TG2.[8] Another prominent strategy is to hydrolyze gluten by exogenous enzyme supplements designed for this purpose. This promising strategy is based on the finding that distinct peptidases or their combinations from different sources are able to hydrolyze gliadin and gliadin peptides *in vitro*.[3,9] Indeed, data are available to indicate that distinct peptidase is capable of accelerating the degradation of gluten in the gastrointestinal system, closely mimicking *in vivo* digestion and suggesting that gluten toxicity might be eliminated by such an approach.[10]

Regardless of the progress made in understanding the pathogenesis of celiac disease, the most conspicuous limitation in the field is the lack of a functional disease-specific animal model. Such a model would also be highly appreciated among researchers developing novel treatment strategies for the disease, as the initial testing of novel drug candidates could then be conducted in animals. A number of attempts have been made to create an animal model for celiac disease by various approaches. These include the generation of transgenic mice expressing the celiac-type human HLA DQ2 or DQ8[11–14] and introduction of celiac-type antibodies in mice,[15–17] but none of the animals used have developed a full-blown villous atrophy together with crypt hyperplasia that are characteristic for untreated celiac disease. Gluten-dependent small-intestinal mucosal damage can be induced in mice[18] and has been described in rhesus macaques,[19] but these two models are not dependent on celiac-type HLA and their applicability as models for celiac disease has thus to be considered with caution. Very recently, DePaolo *et al*.[20] reported that gliadin-fed humanized HLA-DQ8 mice that overexpress IL-15 in the lamina propria (DQ8-D^d-IL-15 transgenic mice) present with a condition resembling early developing celiac disease, where intestinal inflammation is present but the mucosal architecture remains normal. This promising model may prove useful in the future, although its applicability needs to be verified in further studies.

Regardless of numerous attempts, the animal model for celiac disease that reproduces all the aspects of the disorder still awaits development. However, the use of *in vitro* approaches including a variety of cell lines and the celiac patient small-bowel mucosal biopsy organ culture has generated knowledge about pathogenesis of celiac disease and enabled the initial testing of novel treatment options for celiac disease. This review describes the cell-based and organ culture methods widely used in celiac disease and summarizes the major findings obtained with these systems.

Epithelial cell culture models

After ingestion and progress to the small intestine, the gluten-derived gliadin peptides come into contact with the small-bowel mucosal epithelium. In untreated celiac disease, small-bowel mucosal epithelial cells proliferate more rapidly without differentiating to enterocytes, and their turnover by apoptosis is augmented. Moreover, the epithelial junctional integrity is compromised[21,22] and the gliadin peptides therefore gain access to the lamina propria. However, it is not well understood how gluten-derived gliadin peptides modulate the biology of the epithelium and how they cross the epithelial barrier prior to substantial mucosal remodeling during the early stages of the disorder. For all of the above-mentioned reasons, study of the intestinal epithelium in the context of celiac disease is thus manifestly relevant. There are several intestinal epithelial cell lines available, but two of those mostly used in celiac disease research are described here.

T84 cells

T84 cells are derived from a lung metastasis of a colon cancer, and the cell line was first established as a model for studying intestinal epithelial chloride transport. When grown on permeable supports, the cells form confluent polarized layers with high transepithelial resistance, and they evince a chloride secretory response to different chemicals.[23] In addition, the polarized T84 cell layers are characterized with well-delineated intracellular junctions including circumferential tight junctions.[24] For the above-mentioned reason, the cell line, although of colonic origin, is widely used in studying intestinal epithelial permeability to macromolecules and ions even though the cells do not present with features of intestinal enterocyte-type differentiation in such cultures. However, when T84 cells are cultured three-dimensionally in collagen in the presence of either fibroblasts or transforming growth factor β, they differentiate to intestinal enterocytes as assessed by morphological and biochemical criteria.[25]

T84 cells have been applied in research into celiac disease pathogenesis, for instance in studying the innate immune reactions elicited by the toxic gliadin peptides. It has been shown that the toxic gliadin peptide p31–43 creates cellular stress by inducing the production of reactive oxygen species[26] and Fas-dependent apoptosis.[27] Furthermore, it has been shown that untreated celiac disease patients' serum antibodies induce proliferation and reduce differentiation of the three-dimensionally cultured T84 cells[28] and increase the permeability of the T84 cell epithelial monolayer.[29] Moreover, Bethune *et al*.[30] have demonstrated that interferon (IFN)-γ derived from gluten-stimulated celiac patient–specific intestinal T-cells enhances the passage of gluten through the T84 epithelial layer.

Caco-2 cells

The Caco-2 cell line was originally obtained from a relatively well-differentiated human colon adenocarcinoma. When reaching confluence, the Caco-2 cells differentiate

spontaneously.[31] In such cultures, the cells are polarized and possess an ultrastructural morphology of differentiated enterocytes with an apical surface covered by microvilli.[32] In the monolayer, the cells are joined together through apical junctional complexes which include tight junctions and desmosomes.[33] Postconfluent cultures are hallmarked by the emergence of domes in which cells express sucrase isomaltase, alkaline phosphatase and aminopeptidase N, enzymes typical for differentiated enterocytes.[33] Owing to the above-mentioned characteristics, the Caco-2 cell line is widely used as a model for the intestinal epithelial barrier. Caco-2 cells probably comprise the most extensively used epithelial cell line in studies related to celiac disease.

Gliadin or distinct gliadin peptides are reported to induce actin rearrangement,[34] proliferation,[34,35] intracellular oxidative imbalance[36] and apoptosis in Caco-2 cells.[37,38] Gliadin also inhibits the spontaneous differentiation of these cells.[39] Such findings suggest that the celiac disease-inducing agent gliadin itself could at least partly account for the behavioral abnormalities of the small bowel epithelial cells in celiac disease.

In view of the importance of the compromised small-bowel epithelial barrier function in celiac disease pathogenesis, researchers have used the Caco-2 cells in studies seeking to establish which factors could account for this. At least gliadin and two proinflammatory cytokines abundantly expressed in celiac disease, IFN-γ and tumor necrosis factor α, have been reported to affect the expression of several epithelial junctional proteins (for example occludin and ZO-1) and permeability in Caco-2 cells.[40–48]

Caco-2 cells have also been used in studies clarifying how individual gliadin peptides are processed in intestinal epithelial cells. Using Caco-2 cells it has been found that both the toxic p31–43 and the immunodominant p57–68 gliadin peptides are taken up by the cells and interact with the endocytosis compartment.[49] However, the toxic and the immunodominant gliadin peptides seem to be differentially processed in Caco-2 cells,[49] but at least the immunodominant 33-mer gliadin peptide eventually crosses the epithelial barrier to the luminal side.[50] These findings in Caco-2 cells, together with others made in celiac patient biopsies or mucosal organ cultures, have considerably increased our understanding of gliadin processing in intestinal epithelial cells, this presumably being crucial for our understanding of celiac disease pathogenesis.

The epithelial modulating properties of yet another factor possibly participating in celiac disease pathogenesis, namely celiac disease antibodies, have also been studied in Caco-2 cells. It has been shown that celiac patient antibodies induce actin remodeling,[51] interfere with the uptake of p31–43[52] and modify its intracellular transport,[53] and further induce proliferation in Caco-2 cells.[51,54] Moreover, celiac patient IgA has been reported to increase the epithelial permeability of both toxic (p31–43) and immunodominant (p57–68) gliadin peptides.[55]

The Caco-2 cell line has also been used fairly extensively in initial testing of novel treatment options for celiac disease. The cell line has been used in studying the potency of at least a tight junction modulator, zonulin antagonist, Bifidobacteria, germinating cereal enzymes and polymeric binders of gliadin as putative future treatment options.[41–43,45,56–59] All the studies in question demonstrated protective effects of the tested compounds against gluten-induced effects in Caco-2 cells, but their efficacy obviously need to be tested in other systems as well as in patients. Taken together, the use of Caco-2 cells in celiac disease research has shed significant light on the disease pathogenesis and the proof-of-principle efficacy of novel treatment options.

Celiac patient-derived T-cell lines and clones

The extensive evidence that CD4+ T-cells, associated with recognition of HLA DQ2 and DQ8 molecules, are markedly involved in the pathogenesis of celiac disease has inspired researchers to develop *in vitro* T-cell models. The celiac disease-specific T-cells, raised against gluten, have been obtained both from the small-intestinal mucosa[60–62] and the peripheral blood[63–65] of patients suffering from celiac disease. The HLA-DQ2 or DQ8 restricted T-cells isolated from small-bowel mucosal biopsies are typically cultured *in vitro* in the presence of stimulating gluten peptides (T-cell lines), whereafter single, gluten-reactive monoclonal cells (T-cell clones) can be further isolated and maintained in the culture conditions. In contrast, peripheral blood mononuclear cells are isolated from treated celiac disease patient's serum after a short-term *in vivo* gluten challenge.[66] In both cases, activation of T-cells is typically presented as an increase in cell proliferation and IFN-γ-predominant cytokine production.[62,64,67]

Most of the gut-derived T-cells do not recognize native gluten peptides unless they are deamidated by tissue transglutaminase,[6,7] a phenomenon which is thought to occur also in *in vivo* conditions. The majority of studies conducted with celiac patient T-cells have concentrated on identification of gliadin epitopes such as diverse epitopes found in immunodominant α-gliadin 33-mer;[3,68] however, intestinal T-cell lines and clones have also been shown to recognize deamidated rye secalin and barley hordein peptides.[69,70] T-cell lines and clones have been especially useful in the systematic characterization of T-cell reactive gluten epitopes in wheat, rye and barley.[71–73] However, patients seem to respond to distinct gluten peptides,[74,75] resulting in limitations when investigating the overall toxicity of gluten peptides. Application of several simultaneous T-cell lines from different patients is thus probably needed to overcome these challenges.

Similarly to intestinal epithelial cells, celiac patient T-cell lines and clones have been used in testing newly developed treatment forms. For instance, testing of different types of modified gliadin or gluten peptides[76–79] and enzyme therapy[3,42,80–83] as a potential novel treatment form has been carried out in celiac patient T-cell lines or clones.

Small-bowel mucosal biopsy organ culture

The culturing of small-intestinal mucosal biopsies derived *ex vivo* from celiac disease patients was originally introduced by Browning and Trier.[84] The biopsies can be kept

viable in the organ culture system for 24–48 h[85] or even longer, although there is no guarantee of the quality of mucosal morphology after a long-term culture.[86] In preliminary studies, the organ culture method was used in an opposite manner to the current practice. Culture conditions were thought to mimic a gluten-free diet, so that the small-bowel mucosal morphology improved after gluten was excluded.[85–88] In contrast, the present organ culture system is generated to illustrate the harmful effects of gluten, gliadin or distinct gliadin peptides added to the culture supernatant (Figure 1). Since the *ex vivo* biopsy culture contains a wide variety of cell types expressed in the intestinal mucosa *in vivo*, the method allows researchers to investigate an extensive range of markers for gluten toxicity, all the way from the epithelium to activation of immune components in the mucosal *lamina propria*. One of the versatile benefits of the method lies in the fact that various features characteristic for the disorder can be reproduced in biopsies from treated celiac disease patients, thus allowing researchers to find mechanisms related to the development of celiac disease from a healthy mucosa into the active phase of the disease. In addition, some researchers have suggested the organ culture method even as a potential tool for celiac disease diagnosis.[89,90]

As the small-bowel mucosal biopsy organ culture system has been used extensively in studies aiming to clarify the pathogenesis of celiac disease, it would be impossible to summarize the entire literature in this review. However, since biopsies contain a number of different cell types, the issues open to investigation in the system are numerous. For instance, celiac disease researchers have studied the immune mechanisms[4] as well as the contribution of different cytokines[91] in this context. As an example, one of the major findings related to celiac disease pathogenesis, namely the importance of IL-15 during the disease process, was obtained from studies conducted with the organ culture.[5,92–96]

Considering that the small-bowel organ culture method has been widely used in studies aiming to clarify the pathogenesis of celiac disease, it is surprising that the method has only been used in a few studies related to novel treatment forms. To our knowledge, it has only been applied in studying the therapeutic potential of a particular zonulin antagonist[45] and gliadin-degrading proteolytic enzymes,[42,81,97] with promising results.

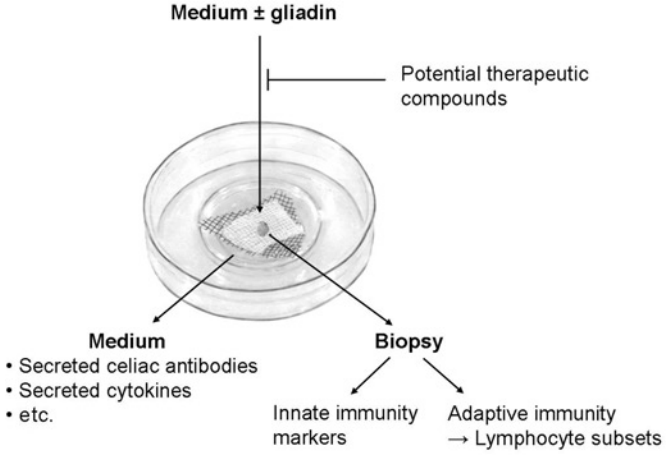

Figure 1 The principle of the small-bowel mucosal organ culture system

Conclusions

In the absence of an animal model for celiac disease, researchers have used different cell lines and the celiac patient small-bowel mucosal organ culture method in studying different aspects of celiac disease pathogenesis and proof-of-concept testing of novel treatment options which could in the future replace the only effective treatment for celiac disease, the gluten-free diet. Although widely used, all of the models presented in this review have their limitations and thus do not always correlate with the circumstances in the human small intestine *in vivo*. For example, the epithelial cell lines are not derived from celiac disease patients but instead are of cancerous origin. Moreover, the epithelial cells and celiac patient T-cell lines and clones lack connection to other cell types present in the intestine. In addition, the small-bowel organ culture system lacks circulation, nervous system and connection to lymphatic organs. Furthermore, the organ culture system is not a high-throughput method. In the future it would thus be worthwhile to develop novel and improved culture systems and to promote celiac disease-specific models. However, even regardless of the above-mentioned shortcomings, researchers have gained important information and been able to add pieces to the celiac disease pathogenesis puzzle using the models described in this review and also other available model systems. Moreover, the studies clarifying the efficacy of novel treatment options carried out with the models described here have yielded encouraging results and some potentially therapeutic compounds have already entered phase II clinical trials.

Author contributions: KL, TR, SS, MM and KK all participated in the writing of the manuscript, and TR made the figure.

ACKNOWLEDGEMENTS

The Celiac Disease Study Group have been financially supported by the Academy of Finland, the Sigrid Juselius Foundation, the Foundation for Pediatric Research, the Pirkanmaa Cultural Foundation, the Competitive Research Funding of Tampere University Hospital, the Research Fund of the Finnish Celiac Society and the European Commission IAPP grant TRANSCOM (contract number PIA-GA-2010-251506).

REFERENCES

1 Lindfors K, Mäki M, Kaukinen K. Transglutaminase 2-targeted autoantibodies in celiac disease: pathogenetic players in addition to diagnostic tools? *Autoimmun Rev* 2010;**9**:744–9
2 Hausch F, Shan L, Santiago NA, Gray GM, Khosla C. Intestinal digestive resistance of immunodominant gliadin peptides. *Am J Physiol Gastrointest Liver Physiol* 2002;**283**:G996–G1003
3 Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C. Structural basis for gluten intolerance in celiac sprue. *Science* 2002;**297**:2275–9

4 Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S, Picard J, Osman M, Quaratino S, Londei M. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet* 2003;**362**:30–7

5 Hüe S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J, Verkarre V, Fodil N, Bahram S, Cerf-Bensussan N, Caillat-Zucman S. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* 2004;**21**:367–7

6 Sjöström H, Lundin KE, Molberg O, Körner R, McAdam SN, Anthonsen D, Quarsten H, Norén O, Roepstorff P, Thorsby E, Sollid LM. Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand J Immunol* 1998;**48**:111–5

7 Molberg O, McAdam SN, Korner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Norén O, Roepstorff P, Lundin KE, Sjöström H, Sollid LM. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med* 1998;**4**:713–7

8 Sollid LM, Khosla C. Novel therapies for coeliac disease. *J Intern Med* 2011;**269**:604–13

9 Gass J, Ehren J, Strohmeier G, Isaacs I, Khosla C. Fermentation, purification, formulation, and pharmacological evaluation of a prolyl endopeptidase from *Myxococcus xanthus*: implications for Celiac Sprue therapy. *Biotechnol Bioeng* 2005;**92**:674–84

10 Mitea C, Havenaar R, Drijfhout JW, 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

11 D'Arienzo R, Stefanile R, Maurano F, Luongo D, Bergamo P, Mazzarella G, Troncone R, Auricchio S, David C, Rossi M. A deregulated immune response to gliadin causes a decreased villus height in DQ8 transgenic mice. *Eur J Immunol* 2009;**39**:3552–61

12 de Kauwe AL, Chen Z, Anderson RP, Keech CL, Price JD, Wijburg O, Jackson DC, Ladhams J, Allison J, McCluskey J. Resistance to celiac disease in humanized HLA-DR3-DQ2-transgenic mice expressing specific anti-gliadin CD4+ T cells. *J Immunol* 2009;**182**: 7440–50

13 Senger S, Maurano F, Mazzeo MF, Gaita M, Fierro O, David CS, Troncone R, Auricchio S, Siciliano RA, Rossi M. Identification of immunodominant epitopes of alpha-gliadin in HLA-DQ8 transgenic mice following oral immunization. *J Immunol* 2005;**175**:8087–95

14 Du Pré MF, Kozijn AE, van Berkel LA, Ter Borg MN, Lindenbergh-Kortleve D, Jensen LT, Kooy-Winkelaar Y, Koning F, Boon L, Nieuwenhuis EE, Sollid LM, Fugger L, Samsom JN. Tolerance to ingested deamidated gliadin in mice is maintained by splenic, type 1 regulatory T cells. *Gastroenterology* 2011;**141**:610–20

15 Smart CJ, Trejdosiewicz LK, Howdle PD. Specific circulating anti-gliadin IgG-class antibody does not mediate intestinal enteropathy in gliadin-fed mice. *Int Arch Allergy Immunol* 1992;**97**:160–6

16 Freitag T, Schulze-Koops H, Niedobitek G, Melino G, Schuppan D. The role of the immune response against tissue transglutaminase in the pathogenesis of coeliac disease. *Autoimmun Rev* 2004;**3**:12–20

17 Di Niro R, Sblattero D, Florian F, Stebel M, Zentilin L, Giacca M, Villanacci V, Galletti A, Not T, Ventura A, Marzari R. Anti-idiotypic response in mice expressing human autoantibodies. *Mol Immunol* 2008;**45**:1782–91

18 Freitag TL, Rietdijk S, Junker Y, Popov Y, Bhan AK, Kelly CP, Terhorst C, Schuppan D. Gliadin-primed CD4+CD45RBlowCD25− T cells drive gluten-dependent small intestinal damage after adoptive transfer into lymphopenic mice. *Gut* 2009;**58**:1597–605

19 Bethune MT, Borda JT, Ribka E, Liu MX, Phillippi-Falkenstein K, Jandacek RJ, Doxiadis GG, Gray GM, Khosla C, Sestak K. A non-human primate model for gluten sensitivity. *PLoS One* 2008;**3**:e1614

20 DePaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W, Marietta EV, Kasarda DD, Waldmann TA, Murray JA, Semrad C, Kupfer SS, Belkaid Y, Guandalini S, Jabri B. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature* 2011;**471**:220–4

21 Schulzke JD, Schulzke I, Fromm M, Riecken EO. Epithelial barrier and ion transport in coeliac sprue: electrical measurements on intestinal aspiration biopsy specimens. *Gut* 1995;**37**:777–82

22 Schulzke JD, Bentzel CJ, Schulzke I, Riecken EO, Fromm M. Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. *Pediatr Res* 1998;**43**:435–41

23 Dharmsathaphorn K, McRoberts JA, Mandel KG, Tisdale LD, Masui H. A human colonic tumor cell line that maintains vectorial electrolyte transport. *Am J Physiol Gastrointest Liver Physiol* 1984;**246**:G204–8

24 Madara JL, Stafford J, Dharmsathaphorn K, Carlson S. Structural analysis of a human intestinal epithelial cell line. *Gastroenterology* 1987;**92**:1133–45

25 Halttunen T, Marttinen A, Rantala I, Kainulainen H, Mäki M. Fibroblasts and transforming growth factor beta induce organization and differentiation of T84 human epithelial cells. *Gastroenterology* 1996;**111**:1252–62

26 Luciani A, Villella VR, Vasaturo A, Giardino I, Pettoello-Mantovani M, Guido S, Cexus ON, Peake N, Londei M, Quaratino S, Maiuri L. Lysosomal accumulation of gliadin p31–43 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa. *Gut* 2010;**59**:311–9

27 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

28 Halttunen T, Mäki M. Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. *Gastroenterology* 1999;**116**:566–72

29 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

30 Bethune MT, Siegel M, Howles-Banerji S, Khosla C. Interferon-gamma released by gluten-stimulated celiac disease-specific intestinal T cells enhances the transepithelial flux of gluten peptides. *Pharmacol Exp Ther* 2009;**329**:657–68

31 Zweibaum A, Triadou N, Kedinger M, Augeron C, Robine-Léon S, Pinto M, Rousset M, Haffen K. Sucrase-isomaltase: a marker of foetal and malignant epithelial cells of the human colon. *Int J Cancer* 1983;**32**:407–12

32 Bolte G, Wolburg H, Beuermann K, Stocker S, Stern M. Specific interaction of food proteins with apical membranes of the human intestinal cell lines Caco-2 and T84. *Clin Chim Acta* 1998;**270**:151–67

33 Pinto M, Robine-Leon S, Appay M-D, Kedinger M, Traidou N, Dussaulx E, Lacroix B, Simon Assmann P, Haffen K, Fog J, Zweibaum A. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line CaCo-2 in culture. *Biol Cell* 1983;**47**:323–30

34 Barone MV, Gimigliano A, Castoria G, Paolella G, Maurano F, Paparo F, Maglio M, Mineo A, Miele E, Nanayakkara M, Troncone R, Auricchio S. Growth factor-like activity of gliadin, an alimentary protein: implications for coeliac disease. *Gut* 2007;**56**:480–8

35 Barone MV, Zanzi D, Maglio M, Nanayakkara M, Santagata S, Lania G, Miele E, Ribecco MT, Maurano F, Auricchio R, Gianfrani C, Ferrini S, Troncone R, Auricchio S. Gliadin-mediated proliferation and innate immune activation in celiac disease are due to alterations in vesicular trafficking. *PLoS One* 2011;**6**:e17039

36 Rivabene R, Mancini E, De Vincenzi M. *In vitro* cytotoxic effect of wheat gliadin-derived peptides on the Caco-2 intestinal cell line is associated with intracellular oxidative imbalance: implications for coeliac disease. *Biochim Biophys Acta* 1999;**1453**:152–60

37 Giovannini C, Sanchez M, Straface E, Scazzocchio B, Silano M, De Vincenzi M. Induction of apoptosis in caco-2 cells by wheat gliadin peptides. *Toxicology* 2000;**145**:63–71

38 Sakly W, Thomas V, Quash G, El Alaoui S. A role for tissue transglutaminase in alpha-gliadin peptide cytotoxicity. *Clin Exp Immunol* 2006;**146**:550–8

39 Giovannini C, Maiuri L, De Vincenzi M. Cytotoxic effect of prolamin-derived peptides on *in vitro* cultures of cell line Caco-2: implications for coeliac disease. *Toxicol In Vitro* 1995;**9**:251–5

40 Sander GR, Cummins AG, Henshall T, Powell BC. Rapid disruption of intestinal barrier function by gliadin involves altered expression of apical junctional proteins. *FEBS Lett* 2005;**579**:4851–5

41 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
42 Stenman S, Venäläinen JI, Lindfors K, Auriola S, Mauriala T, Kaukovirta-Norja A, Jantunen A, Laurila K, Qiao SW, Sollid LM, Männisto PT, Kaukinen K, Mäki M. Enzymatic detoxification of gluten by germinating wheat proteases: implications for new treatment of celiac disease. *Ann Med* 2009;**41**:390–400
43 Stenman S, Lindfors K, Venäläinen JI, Hautala A, Männistö PT, Garcia-Horsman JA, Kaukovirta-Norja A, Auriola S, Mauriala T, Mäki M, Kaukinen K. Degradation of coeliac disease-inducing rye secalin by germinating cereal enzymes: diminishing toxic effects in intestinal epithelial cells. *Clin Exp Immunol* 2010;**161**:242–9
44 Juuti-Uusitalo K, Lindfors K, Mäki M, Patrikainen M, Isola J, Kaukinen K. Inhibition of epithelial growth factor receptor signalling does not preserve epithelial barrier function after *in vitro* gliadin insult. *Scand J Gastroenterol* 2009;**44**:820–5
45 Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, Sapone A, Thakar M, Iacono G, Carroccio A, D'Agate C, Not T, Zampini L, Catassi 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
46 Perry I, Tselepis C, Hoyland J, Iqbal TH, Scott D, Sanders SA, Cooper BT, Jankowski JA. Reduced cadherin/catenin complex expression in celiac disease can be reproduced *in vitro* by cytokine stimulation. *Lab Invest* 1999;**79**:1489–99
47 Ciccocioppo R, Finamore A, Ara C, Di Sabatino A, Mengheri E, Corazza GR. Altered expression, localization, and phosphorylation of epithelial junctional proteins in celiac disease. *Am J Clin Pathol* 2006;**125**:502–11
48 Tripathi A, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnett S, Buzza MS, Antalis TM, Vogel SN, Zhao A, Yang S, Arrietta MC, Meddings JB, Fasano A. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. *Proc Natl Acad Sci* 2009;**106**:16799–804
49 Barone MV, Nanayakkara M, Paolella G, Maglio M, Vitale V, Troiano R, Ribecco MT, Lania G, Zanzi D, Santagata S, Auricchio R, Troncone R, Auricchio S. Gliadin peptide P31–43 localises to endocytic vesicles and interferes with their maturation. *PLoS One* 2010;**5**:e12246
50 Schumann M, Richter JF, Wedell I, Moos V, Zimmermann-Kormann M, Schneider T, Daum S, Zeitz M, Fromm M, Schulzke JD. Mechanisms of epithelial translocation of the alpha2-gliadin-33mer in coeliac sprue. *Gut* 2008;**57**:747–54
51 Barone MV, Caputo I, Ribecco MT, Maglio M, Marzari R, Sblattero D, Troncone R, Auricchio S, Esposito C. Humoral immune response to tissue transglutaminase is related to epithelial cell proliferation in celiac disease. *Gastroenterology* 2007;**132**:1245–53
52 Caputo I, Barone MV, Lepretti M, Martucciello S, Nista I, Troncone R, Auricchio S, Sblattero D, Esposito C. Celiac anti-tissue transglutaminase antibodies interfere with the uptake of alpha gliadin peptide 31–43 but not of peptide 57–68 by epithelial cells. *Biochim Biophys Acta* 2010;**1802**:717–27
53 Lübbing N, Barone MV, Rudloff S, Troncone R, Auricchio S, Zimmer KP. Correction of gliadin transport within enterocytes through celiac disease serum. *Pediatr Res* 2011;**70**:357–62
54 Ferrara F, Quaglia S, Caputo I, Esposito C, Lepretti M, Pastore S, Giorgi R, Martelossi S, Dal Molin G, Di Toro N, Ventura A, Not T. Anti-transglutaminase antibodies in non-coeliac children suffering from infectious diseases. *Clin Exp Immunol* 2010;**159**:217–23
55 Rauhavirta T, Qiao SW, Jiang Z, Myrsky E, Loponen J, Korponay-Szabó IR, Salovaara H, Garcia-Horsman JA, Venäläinen J, Männistö PT, Collighan R, Mongeot A, Griffin M, Mäki M, Kaukinen K, Lindfors K. Epithelial transport and deamidation of gliadin peptides: a role for coeliac disease patient immunoglobulin A. *Clin Exp Immunol* 2011;**164**:127–36
56 Laparra JM, Sanz Y. Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion. *J Cell Biochem* 2010;**109**:801–7
57 De Palma G, Cinova J, Stepankova R, Tuckova L, Sanz Y. Pivotal Advance: Bifidobacteria and Gram-negative bacteria differentially influence immune responses in the proinflammatory milieu of celiac disease. *J Leuk Biol* 2010;**87**:765–78
58 Olivares M, Laparra M, Sanz Y. Influence of *Bifidobacterium longum* CECT 7347 and gliadin peptides on intestinal epithelial cell proteome. *J Agric Food Chem* 2011;**59**:7666–71
59 Pinier M, Verdu EF, Nasser-Eddine M, David CS, Vézina A, Rivard N, Leroux JC. Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium. *Gastroenterology* 2009;**136**:288–98
60 van de Wal Y, Kooy YM, van Veelen PA, Pena SA, Mearin LM, Molberg O, Lundin KE, Sollid LM, Mutis T, Benckhuijsen WE, Drijfhout JW, Koning F. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc Natl Acad Sci USA* 1998;**95**:10050–4
61 Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. Gliadin-specific, HLA-DQ(alpha 1*0501, beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med* 1993;**178**:187–96
62 Ellis HJ, Pollock EL, Engel W, Fraser JS, Rosen-Bronson S, Wieser H, Ciclitira PJ. Investigation of the putative immunodominant T cell epitopes in coeliac disease. *Gut* 2003;**52**:212–7
63 Ráki M, Fallang LE, Brottveit M, Bergseng E, Quarsten H, Lundin KE, Sollid LM. Tetramer visualization of gut-homing gluten-specific T cells in the peripheral blood of celiac disease patients. *Proc Natl Acad Sci* 2007;**104**:2831–6
64 Anderson RP, van Heel DA, Tye-Din JA, Barnardo M, Salio M, Jewell DP, Hill AV. T cells in peripheral blood after gluten challenge in coeliac disease. *Gut* 2005;**54**:1217–23
65 Gjertsen HA, Lundin KE, Sollid LM, Eriksen JA, Thorsby E. T cells recognize a peptide derived from alpha-gliadin presented by the celiac disease-associated HLA-DQ (alpha 1*0501, beta 1*0201) heterodimer. *Hum Immunol* 1994;**39**:243–52
66 Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AV. *In vivo* antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. *Nat Med* 2000;**6**:337–42
67 Nilsen EM, Gjertsen HA, Jensen K, Brandtzaeg P, Lundin KE. Gluten activation of peripheral blood T cells induces a Th0-like cytokine pattern in both coeliac patients and controls. *Clin Exp Immunol* 1996;**103**:295–303
68 Qiao SW, Bergseng E, Molberg Ø, Xia J, Fleckenstein B, Khosla C, Sollid LM. Antigen presentation to celiac lesion-derived T cells of a 33-mer gliadin peptide naturally formed by gastrointestinal digestion. *J Immunol* 2004;**173**:1757–62
69 Vader LW, Stepniak DT, Bunnik EM, Kooy YM, de Haan W, Drijfhout JW, Van Veelen PA, Koning F. Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology* 2003;**125**:1105–13
70 Kilmartin C, Wieser H, Abuzakouk M, Kelly CP, Jackson J, Feighery C. Intestinal T cell responses to cereal proteins in celiac disease. *Dig Dis Sci* 2006;**51**:2002–9
71 Beissbarth T, Tye-Din JA, Smyth GK, Speed TP, Anderson RP. A systematic approach for comprehensive T-cell epitope discovery using peptide libraries. *Bioinformatics* 2005;**21**:i29-i37
72 Arentz-Hansen H, McAdam SN, Molberg Ø, Fleckenstein B, Lundin KE, Jørgensen TJ, Jung G, Roepstorff P, Sollid LM. Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues. *Gastroenterology* 2002;**123**:803–9
73 Shan L, Qiao SW, Arentz-Hansen H, Molberg Ø, Gray GM, Sollid LM, Khosla C. Identification and analysis of multivalent proteolytically resistant peptides from gluten: implications for celiac sprue. *J Proteome Res* 2005;**4**:1732–41
74 Vader W, Kooy Y, Van Veelen P, De Ru A, Harris D, Benckhuijsen W, Peña S, Mearin L, Drijfhout JW, Koning F. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology* 2002;**122**:1729–37
75 Lundin KE, Sollid LM, Anthonsen D, Noren O, Molberg O, Thorsby E, Sjöström H. Heterogeneous reactivity patterns of HLA-DQ-restricted, small intestinal T-cell clones from patients with celiac disease. *Gastroenterology* 1997;**112**:752–9
76 Gianfrani C, Siciliano RA, Facchiano AM, Camarca A, Mazzeo MF, Costantini S, Salvati VM, Maurano F, Mazzarella G, Iaquinto G, Bergamo P, Rossi M. Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease. *Gastroenterology* 2007;**133**:780–9
77 Huan J, Meza-Romero R, Mooney JL, Vandenbark AA, Offner H, Burrows GG. Single-chain recombinant HLA-DQ2.5/peptide molecules

block α2-gliadin-specific pathogenic CD4+ T-cell proliferation and attenuate production of inflammatory cytokines: a potential therapy for celiac disease. *Mucosal Immunol* 2011;**4**:112–20
78 Kapoerchan VV, Wiesner M, Overhand M, van der Marel GA, Koning F, Overkleeft HS. Design of azidoproline containing gluten peptides to suppress CD4+ T-cell responses associated with celiac disease. *Bioorg Med Chem* 2008;**16**:2053–62
79 Xia J, Bergseng E, Fleckenstein B, Siegel M, Kim CY, Khosla C, Sollid LM. Cyclic and dimeric gluten peptide analogues inhibiting DQ2-mediated antigen presentation in celiac disease. *Bioorg Med Chem* 2007;**15**:6565–73
80 Gass J, Bethune MT, 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
81 Marti T, Molberg O, Li Q, Gray GM, Khosla C, Sollid LM. Prolyl endopeptidase-mediated destruction of T cell epitopes in whole gluten: chemical and immunological characterization. *J Pharmacol Exp Ther* 2005;**312**:19–26
82 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–G29
83 Siegel M, Bethune MT, Gass J, Ehren J, Xia J, Johannsen A, Stuge TB, Gray GM, Lee PP, Khosla C. Rational design of combination enzyme therapy for celiac sprue. *Chem Biol* 2006;**13**:649–58
84 Browning TH, Trier JS. Organ culture of mucosal biopsies of human small intestine. *J Clin Invest* 1969;**48**:1423–32
85 Jos J, Lenoir G, Ritis GD, Rey J. *In vitro* pathogenetic studies of coeliac disease. Effects of protein digests on coeliac intestinal biopsy specimens maintained in culture for 48 hours. *Scand J Gastroenterol* 1975;**10**:121–8
86 Falchuk ZM, Gebhard RL, Sessoms C, Strober W. An *in vitro* model of gluten-sensitive enteropathy. Effect of gliadin on intestinal epithelial cells of patients with gluten-sensitive enteropathy in organ culture. *J Clin Invest* 1974;**53**:487–500
87 Hauri HP, Kedinger M, Haffen K, Gaze H, Hadorn B, Hekkens W. Re-evaluation of the technique of organ culture for studying gluten toxicity in coeliac disease. *Gut* 1978;**19**:1090–8
88 Fluge G, Aksnes L. Morphological and morphometric assessment of human duodenal biopsies maintained in organ culture. *In vitro* influences of gluten in coeliac disease. *Scand J Gastroenterol* 1981;**16**:555–67
89 Bonamico M, Sabbatella L, Di Tola M, Vetrano S, Ferri M, Nenna R, Mariani P, Picarelli A. Antiendomysial antibody detection in biopsy culture allows avoidance of gluten challenge in celiac children. *J Pediatr Gastroenterol Nutr* 2005;**40**:165–9
90 Picarelli A, Di Tola M, Sabbatella L, Anania MC, Calabro A, Renzi D, Bai JC, Sugai E, Carroccio A, Di Prima L, Bardella MT, Barisani D, Ribes-Koninckx C, Aliaga ED, Gasparin M, Bravi E; Multicentre Organ Culture System Study Group. Usefulness of the organ culture system in the *in vitro* diagnosis of coeliac disease: a multicentre study. *Scand J Gastroenterol* 2006;**41**:186–90
91 Fina D, Sarra M, Caruso R, Del Vecchio Blanco G, Pallone F, MacDonald TT, Monteleone G. Interleukin 21 contributes to the mucosal T helper cell type 1 response in coeliac disease. *Gut* 2008;**57**:887–92
92 Maiuri L, Ciacci C, Auricchio S, Brown V, Quaratino S, Londei M. Interleukin 15 mediates epithelial changes in celiac disease. *Gastroenterology* 2000;**119**:996–1006
93 Maiuri L, Ciacci C, Raia V, Vacca L, Ricciardelli I, Raimondi F, Auricchio S, Quaratino S, Londei M. FAS engagement drives apoptosis of enterocytes of coeliac patients. *Gut* 2001;**48**:418–24
94 Zanzi D, Stefanile R, Santagata S, Iaffaldano L, Iaquinto G, Giardullo N, Lania G, Vigliano I, Vera AR, Ferrara K, Auricchio S, Troncone R, Mazzarella G. IL-15 interferes with suppressive activity of intestinal regulatory T cells expanded in celiac disease. *Am J Gastroenterol* 2011;**106**:1308–17
95 Bernardo D, Garrote JA, Allegretti Y, León A, Gómez E, Bermejo-Martin JF, Calvo C, Riestra S, Fernández-Salazar L, Blanco-Quirós A, Chirdo F, Arranz E. Higher constitutive IL15R alpha expression and lower IL-15 response threshold in coeliac disease patients. *Clin Exp Immunol* 2008;**154**:64–73
96 Benahmed M, Meresse B, Arnulf B, Barbe U, Mention JJ, Verkarre V, Allez M, Cellier C, Hermine O, Cerf-Bensussan N. Inhibition of TGF-beta signaling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease. *Gastroenterology* 2007;**132**:994–1008
97 Matysiak-Budnik T, Candalh C, Cellier C, Dugave C, Namane A, Vidal-Martinez T, Cerf-Bensussan N, Heyman M. Limited efficiency of prolyl-endopeptidase in the detoxification of gliadin peptides in celiac disease. *Gastroenterology* 2005;**129**:786–96

(Received September 1, 2011, Accepted October 19, 2011)