Autoimmunity and Celiac Disease

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Abstract: In celiac disease (CD), abnormal immune-mediated responses follow ingestion of gluten. Although the triggering agent is a dietary protein, the disease has autoimmune components because of the presence of autoantibodies and its association with autoimmune conditions. We review the most recent studies on CD pathogenesis and the possibilities to modulate immune dysfunction in CD.

INTRODUCTION

Celiac Disease (CD) is a common disorder of the small intestine characterized by a permanent intolerance to wheat gluten. In CD patients, intolerance to gluten involves both abnormal innate and adaptive immune responses (see below), in addition to humoral dysfunction typified by the presence of antibodies against the self-antigen tissue transglutaminase (tTG) [1]. tTG is a key factor in the pathogenesis of CD since it deamidates specific glutamine residues favoring the recognition of gluten peptides by $CD4^+$ T lymphocytes [2-5].

The isolation of gliadin-specific T cells from CD patients in recent times has significantly contributed to a better understanding of the immune networks responsible for the induction and maintenance of CD [6-10]. At the meantime, the observation of an increased prevalence of autoimmunity in CD patients has underscored the possibility of common abnormalities in the mechanisms of immune homeostasis in CD and other autoimmune diseases. Studies on these aspects of the pathogenesis of CD may unveil important steps in the pathogenesis of this disease if not contributing to a better understanding of some general mechanisms that control (auto)immune responses [11-17]. This review critically highlights and discusses the advances, obstacles and limitations of the most recent studies in this rapidly expanding field.

CELIAC DISEASE: A PATHOGENETIC OVERVIEW

CD is a widespread disorder that affects Caucasian populations including Europeans and North Africans, in addition to North Americans and Australians, as reported in several epidemiologic studies [18-20]. In the individuals with CD, ingestion of wheat gluten and related proteins of other edible cereals, such as rye and barley (and probably also oat), can induce chronic inflammatory lesions that are more frequently confined to the small intestinal mucosa but can also be localized to extra intestinal sites [1,21].

In the last decade, a large body of evidence has elucidated how gluten can activate immune responses in the small intestine that lead to the destruction of the epithelial mucosa and subsequently cause deleterious effects on intestinal absorption of nutrients [1,22], and immune components of CD have been identified.

Both $CD4^+$ and $CD8^+$ T lymphocytes play a crucial role in the inflammatory events that lead to damage of the intestinal mucosa [22]. A role for innate immune cells has also emerged, and it appears that the cytokine IL-15 may be pivotal in the cytotoxic innate immune responses following contact with gluten in the CD patients [23-27].

How can the immune system influence CD? The intestinal tract represents a site where large amounts of antigens pass through every day without immune consequences. In physiologic conditions, T cells circulate in the blood but also migrate into tissues, where they effectively patrol from local invasion of pathogens. In the gastrointestinal tract, T cells are present in the so-called gut-associated lymphoid tissue (GALT). Task of the GALT is to remain immunologically unresponsive (tolerant) to harmless antigens such as food proteins and/or commensal bacteria, and to be able at the same time to promptly mount efficient responses to harmful antigens such as those derived from pathogens [28,29]. This physiologic condition is not present in CD. In the disease, T cells that recognize dietary gluten-derived peptides become activated, proliferate, and secrete interferon-γ (IFN-γ), an inflammatory cytokine with significant cytophatic effects [30]. As if the inflammatory response would not create enough damage to the intestinal mucosa, gluten-derived peptides (i.e. a peptide mapping in the 31-49 region of α-gliadins, the main component of gluten, see below) exert further toxic effects including a mitogen-like activity on epithelial cells and a delay of the epidermal growth factor (EGF) receptor inactivation that causes prolonged EGF activity [31]. As consequence of the EGF activity, in the CD mucosa there is a complex tissue remodeling that also involves the activation of metalloproteinases [32].

CHEMICAL STRUCTURE AND BIOLOGICAL PROPERTIES OF GLUTEN

As discussed above, in CD there is abnormal immune reactivity to gluten (which is a storage protein of wheat seed endosperm). From a nutritional standpoint, gluten is a rather

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Table 1. Immunogenic Peptides of Gliadin

LQLQPFPQP**Q**LPYPQP**Q**LPYPQP**Q**LPYPQPQPF	33-mer α/β-gliadin(57-89) [ref. 42]
P**Q**LPQFLQPQPYPQP**Q**LPYPQPQPF	25-mer α/β-gliadin(64-89) [ref. 41] [a, b]
QPQPYPQP**Q**LPYPQPQPF	18-mer α/β-gliadin(71-89) [ref. 41] [a, b]
PQQQTLQP**Q**QPAQL	14-mer γ-gliadin(105-118) [ref. 41] [a, b]
QLQPFPQP**Q**LPYPQPQP	17-mer α/β-gliadin(57-73) [ref. 77] [a, f, g]
QP**Q**QPFPQP**Q**QPFPWQP	17-mer ω-gliadin(102-118) [ref.] [a, f, g]

Note - **Q**XP: consensus for glutamine deamidation by tTG

poor nutrient that provides elasticity and viscosity to food (i.e. texture and consistency) [33].

Gluten and related proteins of barley and rye are also known as prolamins, for the high content of amino acids such as glutamine (40%) and proline (20%) [34]. According to the molecular weight, gluten is divided into the categories of gliadin (28-75 kDa) and glutenins (45-50 kDa), the latter being divided into low molecular (LMW) and high molecular (HMW) glutenins. Through several covalent bonds between cysteine residues, glutenins polymerize in an elastic matrix that traps gliadins and allows the binding of water, an essential element for viscosity.

Gliadin, the alcohol-soluble fraction of gluten, is a complex mixture of 40 highly homologue proteins that are divided according to their electrophoresis mobility into 3 families: α/β-, γ- and ω-gliadins [33]. A large number of studies, based on reactivity of intestinal T-cell lines and clones from celiac intestine have shown that gliadin contains numerous immunogenic peptides (described in detail in the next paragraph).

Experimental evidence has also indicated a direct, non immune-mediated, cellular toxicity of gliadin-derived peptides *in vitro* on both cell culture systems and mucosal explants. For example, a peptic-tryptic digest of gliadin (PT-gliadin) can agglutinate *in vitro* the K562 (subclone S) cell line [35] and can interfere with the differentiation of fetal rat intestine [36], in addition to being capable of direct damage for the intestinal mucosa [37]. Furthermore, in cultures of celiac small intestinal mucosal tissue – an *in vitro* model for the study of CD - the presence of PT-gliadin or the short peptide 31-43 can specifically prevent a restitution of enterocyte height that would normally occur within 24-48 hours [37]. Finally, PT-gliadin and the 31-43 peptide exert a direct cytotoxic effect on epithelial Caco2 cells through the induction of *fas*-mediated apoptosis [38] and a delayed inactivation of the EGF receptor – phenomena that favor cell growth in the crypts of the atrophic mucosa of CD patients [31].

GLUTEN PEPTIDES STIMULATE IMMUNE RESPONSES

The isolation of T-cells and the establishment of gluten reactive T-cell lines and clones from damaged intestinal CD mucosa have provided valuable tools to identify the peptides of gluten that are involved in the pathogenesis of CD. Peptides that activate the adaptive $CD4^+$ T cell responses have been found in α-gliadins, γ-gliadins [7,8,39,40] and, recently, in ω-gliadins[a].

Despite the recent progress, the identification of all gluten peptides involved in the pathogenesis of CD remains a daunting task because of the great heterogeneity of gluten proteins. Many gliadin epitopes have been identified and purified by high performance liquid chromatography (HPLC) followed by mass spectrometry analysis (Table **1**). In those cases, peptides have been derived from enzymatic digestion of whole or recombinant gliadins or from the screening of large libraries of overlapping peptides [40]. Recently, gluten immunogenic peptides were identified in whole gliadin extracts for their susceptibility to deamidation by tTG using as a probe monodansylcadaverine, a tTG fluorescent substrate (Table **2**) [41][b]. The indirect evidence from these studies, even if informative, may possibly miss some of the complexity of the events that occur *in vivo*. In any case, the data obtained so far indicate that despite a large number of gluten peptides are capable to stimulate T-cell responses in CD mucosa, a single 33-mer peptide has the highest T-cell stimulatory capacity in a large cohort of CD patients [42]. This peptide maps at the level of the 56-88 residues of α-gliadin and harbors six copies of three different T-cell epitopes [42]. Because of the high content of proline in this 33-mer peptide that provides resistance to gastrointestinal enzymatic digestion, the 33-mer peptide can reach immune cells in an intact and highly stimulatory form [42]. Furthermore, in a recent

Table 2. Use of MonoDansylCadaverine (MDC) for Identification of Gluten Peptides Susceptible to tTG Deamidation

Extraction of gliadins from wheat flour
Gliadin digestion with pepsin-trypsin enzymes (PT-gliadin)
Treatment of PT-gliadin with tTG in the presence of MDC
HPLC purification of the PT-gliadin and detection of fluorescent peaks (fluorescent peaks correspond to Q-MDC-tagged gliadin peptides)
Characterization of peptides by mass spectrometry

[a] Camarca, A.; Anderson, R.; Mamone, G.; Rossi, M.; Iaquinto, G.;Giardullo, N.; Auricchio, S.; Troncone, R.; Gianfrani, C. *Gastroenterology* **2006**, A94, 635.

[b] Mamone G, Camarca A. Addeo F, Longobardo L, Ferranti P, Auricchio S, Troncone R, Gianfrani C. *Gastroenterology* **2005,** AGA2005.

study in which intestinal T-cell responses from HLA-DQ2$^+$ CD patients were analyzed toward a panel of 21 different gliadin and glutenin peptides[a], it was found that responses to α-gliadins were mainly focused on peptides mapping to the 56-88 region that had high amino acid sequence similarities. By contrast, the responses to γ-peptides were multiple and reflected amino acid sequence heterogeneity [8,39,40][a], suggesting a more prominent immunological relevance of γ-gliadin peptides in the pathogenesis of CD than what considered so far. Within this context, it must also be noted that although susceptibility to CD seems not to be genetically associated with HLA class-I genes, an α-gliadin-derived peptide (mapping to the 123-132 position) stimulated HLA-A2.1-restricted CD8$^+$ T lymphocytes from both peripheral blood and intestinal mucosa from CD patients [43]. Interestingly, intestinal MHC-A2$^+$ epithelial Caco2 cells pulsed with the p123-132 peptide induced the production of granzyme B and IFN-γ from intestinal CD8$^+$ T cells of CD patients, suggesting that gliadin-specific effector CD8$^+$ T-cells may contribute to the damage of the epithelial cell layer in CD [Mazzarella, G., *et al*., submitted].

INNATE IMMUNITY AND EPITHELIAL DAMAGE

One of the histological characteristics of the celiac intestinal mucosa is a massive infiltration of TCRγ/δ$^+$ and CD8$^+$ TCRα/β$^+$ T-cells. Intraepithelial cell (IEL) infiltration is found in all forms of CD lesions and provides a diagnostic tool in the case of mild forms (partial mucosa atrophy) of CD. The investigation of the role of IEL infiltration in the epithelial cell damage of CD has led to the finding that in the acute disease, following gliadin exposure, enterocytes express the stress molecule MIC(A/B) and induce the activation of intraepithelial, cytotoxic NKG2D CD8$^+$ T cells in a TCR-independent manner. Following stress-induced activation, IEL CD8$^+$ T cells that express NKG2A/D molecules kill the surrounding epithelial cells, causing villous atrophy [26].

Also, a gliadin-dependent, non T-cell-mediated, damage of the integrity of intestinal epithelium in the atrophic CD mucosa is favored by upregulated expression of the cytokine IL-15 [23]. In presence of IL-15, epithelial cells overexpress MICA/B and *fas* molecules and become apoptotic [26,44], suggesting a crucial role of this cytokine in the proinflammatory events associated with CD lesions.

HUMORAL RESPONSES TO TISSUE TRANSGLUTAMINASE TYPE II, A KEY ENZYME IN THE PATHOGENESIS OF CD

Since 1997, when Schuppan and colleagues discovered tissue transglutaminase type II (tTG2) as an autoantigen in CD, significant progress has been done in elucidating the role of tTG2 in the pathogenesis of CD. tTG2 is a ubiquitous Ca^{++}-dependent enzyme whose main physiologic role is the healing of tissue wounds [45,46]. tTG2 catalyses protein cross-linking (transamidation) through lysine and glutamine isopeptide bonds but, in the presence of water, tTG2 catalyses deamidation of specific glutamine (Q) residues to glutamic acid and enhances the stimulatory capacity of gluten-derived peptides by favoring their binding to HLA-DQ2/8 molecules [1,2]. In particular, recent studies have reported that tTG2 preferentially deamidates Q residues corresponding to the QXP consensus sequence [4]. Interestingly, the pronounced susceptibility of gluten for deamidation by tTG represents an important tool to detect the immunologic active gliadin peptides (toxic) that are present in edible cereals [41][b].

From a clinical point of view, IgA antibodies to tTG2 - also known as anti-endomysium antibodies (EMA) for their cross-reactivity with tissue endomysium - have a great diagnostic relevance in CD because of their sensitivity and specificity (about 95%). Moreover, intestinal deposition of anti-tTG2 autoantibodies can possibly represent an early predictor of forthcoming CD, considering that deposits of such antibodies have been found in the intestinal mucosa of subjects with normal villous architecture and with negative serology who subsequently developed CD [47]. Although the anti-tTG/EMA IgA antibody titers correlate with disease status and with ingestion of gluten, it remains unclear how gluten can drive their formation and, more importantly, what is the role of anti-tTG/EMA in the pathogenesis of CD. Furthermore, whether antibodies against tTG2 could represent a secondary mechanism aimed at counteracting the tTG2 deamidase activity in CD mucosa is still controversial [48,49]. However, it is clear that *in vitro* the anti-tTG2 antibodies interfere with the differentiation of epithelial cells, most likely by inhibiting the generation of the active form of TGF-β [50].

In any case, upregulation of tTG2 in inflamed sites may generate a variety of antigenic epitopes by cross linking or deamidating endogenous or exogenously-derived proteins. The unmasking of cryptic epitopes has been hypothesized as a general immune mechanism operating at sites of inflammation, where antigen processing and presentation may be more effective [51]. Some authors have tried to explain why these autoantibodies are dependent on the presence of gluten in the diet by advocating the possibility of a T-cell help for the production of autoantibodies provided by gliadin-specific T cells in the CD mucosa [1]. On the other hand, a recent study has suggested that anti-tTG IgA antibodies of CD patient can cross-react with the protein VP-7 of rotavirus - a very frequent intestinal infectious agent in childhood [52], suggesting the hypothesis that rotavirus infection could help triggering CD *via* mechanisms of molecular mimicry.

In addition to the anti-tTG2 antibodies, other autoantibodies present in sera of CD patients may include antibodies to actin [53] and to calreticulin [54].

EPIDEMIOLOGY, CLINICAL MANIFESTATIONS AND DIAGNOSIS

Following the original description that gluten is the agent that causes CD [55], a better understanding of the clinical aspects of CD and the availability of serological tests to detect antibodies to tTG and gliadin have contributed to a significantly improved diagnosis and management of the disease [56]. Today, CD represents one of the most frequent intestinal disorders. A recent study estimated that about 1:100 of Caucasians are affected by gluten intolerance when including both symptomatic and silent cases [18-20].

Traditionally, the hallmark of the disease is an enteropathy of the small intestine (ranging from mild to severe). Histologically, the mucosa of the small intestinal shows villous

flattening (atrophy) and crypt hypertrophy frequently associated with chronic diarrhea, abdominal pain, and weight loss. Additionally, CD can also present in the form of extra intestinal manifestations such as iron-deficiency anemia, fatigue, infertility, and neurological symptoms [21,57].

GENETIC FACTORS

It is well established that CD has a strong genetic association with both HLA and non-HLA genes [58]. According to recent multi-center studies, almost the totality of CD patients carry either the HLA alleles DQA*0501/DQB*02 (95%) or the HLA allele DQA*0301/DQB*0302 (5%) [59]. Since those two alleles encode respectively the HLA-DQ2 and HLA-DQ8 heterodimers, the presence of alleles encoding the α chain of DQA1*05 and the β chain of DQB1*02 appears mandatory but not sufficient for the development of CD. On the other hand, a DQB*02 gene dosage effect has been reported as an increasing risk factor for CD [60]. This hypothesis is supported by functional studies that indicate a linear correlation between the amount of DQ2 β chain expressed on antigen presenting cells and the dose of gluten peptides presented to cognate Th1 cells [61]. Interestingly, an allele of the MHC class-I chain related gene A (MICA) that encodes for a stress-induced molecule, MICA A5.1, has recently been shown to confer additive genetic effects, particularly in the case of "atypical" silent forms of CD [62].

In regard to non-MHC genes, many studies have indicated that CD relatives - where at least one member of the family is affected - have a prevalence of the disease of about 10% (ten-fold higher than in the general population) [18,57]. Additionally, disease concordance in monozygotic twins is almost 80% [63], suggesting a strong genetic predisposition to CD (even though the lack of absolute concordance indicates an influence by unidentified environmental factors).

Studies of genetic linkage have also identified the chromosomal regions 2q33, 5q31-q33, and 19p13.1 as candidate regions containing genes linked to disease susceptibility [58]. In particular, the interest in a gene encoded within the 2q33 region - CTLA4 - has given contradictory results in linkage studies on different populations [58]. Similarly, conflicting data exists on the genetic association with the myosin IXb gene on chromosome 19, which was described as a gene associated with susceptibility in Dutch patients but that was not confirmed by subsequent studies [64,65].

AUTOIMMUNITY AND CD

CD can be considered an autoimmune disease because of the presence of autoantibodies in both the serum and the intestinal mucosa of CD patients. Although the role of anti-tTG autoantibodies in the celiac intestinal lesions is not clear, the finding has nonetheless important diagnostic relevance - particularly in the cases of mild mucosa lesions. Moreover, serum titers of anti-tTG autoantibodies correlate with the ingestion of gluten in CD patients, and the removal of gluten from diet causes for rapid reversal to low serum levels of these autoantibodies from elevated titers caused by gluten ingestion [66].

Of note, CD also associates with high prevalence of concomitant autoimmune disease (approximately 5-10 times more than in the general population). This aspect could be secondary to a genetic background of the CD patients that is not very efficient in controlling immune tolerance. However, epidemiologic studies indicate that autoimmune diseases associated with CD can be type-1 diabetes, Hashimoto's thyroiditis, and Sjogren's syndrome. An increased frequency of other concomitant conditions such as cardiomyopathy, ataxia, cholangitis and autoimmune hepatitis is also reported [11-17].

An intriguing observation is that the association of CD with other autoimmune diseases is significantly less frequent in those patients diagnosed with CD in the first two years of life [12]. Therefore, it may appear that only a long-time exposure of the immune system to gluten can lead to increased incidence of autoimmunity after a series of events initially triggered by gluten but ultimately involving other mechanisms of immune dysfunction.

A mechanistic correlation between gluten ingestion and autoimmune diseases in CD patients is object of debate [67]. It is also controversial whether a gluten-free diet may promote amelioration of autoimmune disease, particularly type-1 diabetes [68,69], although in the non-obese diabetic mouse (NOD) – an animal model of type-1 diabetes - the withdrawal of gluten from diet reduces the onset of spontaneous autoimmunity [70].

MANAGEMENT OF CD: FROM THE BENCH TO *IN VIVO* THERAPEUTIC INTERVENTION

To date, the only available treatment for CD is the exclusion of gluten from diet. This dietary regimen is safe although poorly compliant. Nevertheless, even after many years of gluten avoidance, CD patients never acquire tolerance to gliadin, and re-exposure to this antigen results in acute disease [71]. This is because the immune regulation during CD is unsuccessful in down-regulating gliadin-induced mucosal inflammatory responses [72]. Therefore, although removal of gluten from diet is effective in restoring a normal intestinal mucosa in CD patients, a pharmacological therapy alternative to a gluten-free diet is in strong demand both for a temporary use and as a complement in the diet [71].

The improved understanding of the mechanisms involved in the pathogenesis of CD has opened several promising scenarios for therapeutic intervention and many therapeutic approaches are under testing in early phases of clinical trials. We discuss below the current molecular and cellular-based approaches to treat CD after briefly reviewing the drugs that target the enzymatic breakdown of gluten.

DRUGS THAT TARGET THE ENZYMATIC BREAKDOWN OF GLUTEN

The recent finding that bacterial prolyl endopeptidases (PEPs) can efficiently degrade gluten peptides by cleaving proline bonds has opened promising perspectives for the therapeutic use of PEPs in CD [42]. However, one limitation in using these proteolytic enzymes for oral therapy is that PEPs are irreversibly inactivated by pepsin and by the acidic pH of the stomach.

Partly overcoming this limitation, it has recently been shown that the treatment of crude wheat gluten with low-pH-

resistant PEPs from *Aspergillus niger* can markedly reduce the stimulatory capacity to gluten from CD-derived T cell lines [73]. Additionally, preliminary *in vivo* studies indicate that oral administration of PEPs concomitantly with wheat baked food can efficiently and rapidly degrade gluten proteins in the proximal gastrointestinal tract[c]. Thus, PEPs may possibly represent a good future therapeutic possibility in CD following more intense investigation.

ENZYMATIC DETOXIFICATION OF WHEAT FLOUR

A very recent study showed the possibility to detoxify gluten by treating wheat flour with transglutaminase and an amine donor such as lysine methyl ester [74]. This enzymatic pre-treatment of wheat flour drastically reduced the immunogenicity of gluten, as demonstrated by its inability to stimulate gliadin-reactive, intestinal T-cell lines after treatment [74].

DRUGS THAT INTERFERE WITH THE INTESTINAL TRANSPORT OF GLUTEN

Events such as stress or pathogens that disrupt the integrity of the intestinal epithelial barrier by disassembling the tight junctions have the potential to increase transport of antigens into the intestinal mucosa. It is hypothesized that an alteration of intestinal permeability can contributes to the onset and/or to maintenance of CD and, more speculatively, of other concomitant autoimmune conditions.

In this context, it is noteworthy that gliadin can induce the release of zonulin, an analogue of the ZOT enterotoxin from *Vibrio cholerae* that increases intestinal permeability [75]. An inhibitor of zonulin, AT-1001, is currently being tested in clinical trials for the treatment of CD patients on a gluten-free diet[d,e].

PEPTIDE THERAPY

One of the most desirable goals for the therapy of CD would be an immune modulation that can target gluten-reactive pathogenic cells without concomitant non-specific suppression. This could be achieved using peptide vaccines, which should provide coverage for the most immunogenic epitopes of the "tolerizing" molecules (in this case, gliadin). As mentioned before, the number of gluten immunogenic peptides identified so far is quite conspicuous and largely heterogeneous[a]. Therefore, the identification of the repertoire of gluten peptides that can induce intestinal lesions in CD is a prerequisite for engineering peptides to use in the therapy of CD.

Encouraging results have come from clinical trials using peptide vaccines in several allergic and autoimmune diseases [76]. In regard to CD, a vaccine with a peptide that combines two immunodominant α- and ϖ- gliadin 17-mer peptides (combo-epitope) is currently under investigation in ongoing clinical trials [77][f,g].

[c] Mitea C. XII International Celiac Disease Symposium, New York, November 2006.

[d] Paterson B. Alba Therapeutics. US patent 6548925 and [c]XII International Celiac Disease Symposium, New York, November **2006**.

[f] Anderson R. granted patent WO/2001/025793 and [g]XII International Celiac Disease Symposium, New York, November **2006**.

CONCLUSIONS

Significant advances have contributed in recent times to a better understanding of the molecular and cellular events involved in the pathogenesis of CD. The presence of autoantibodies to tTG and a better definition of the chemical characteristics of gliadin and its peptides in relation to their interaction with immune-cell molecules have resulted in the design of new strategies of intervention that have a potential to modulate disease activity. While encouraging preliminary results obtained thus far suggest that some approaches may possibly prove beneficial, a significant amount of additional work is required to identify the best targets to have effective therapies for CD.

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ABBREVIATIONS

CD = Celiac disease

tTG = Tissue transglutaminase

REFERENCES

[1] Sollid, L.M. *Nat. Rev. Immunol.,* **2002**, *2*, 647.
[2] Molberg, O.; McAdam, S.N.; Korner, R.; Quarsten, H.; Kristiansen, C.; Madsen, L.; Fugger, L.; Scott, H.; Noren, O.; Roepstorff, P.; Lundin, K.E.A.; Sjorstrom, H.; Sollid, L. *Nat. Med*., **1998**, *4*, 713.
[3] Van de Wal, Y.; Kooy, Y.; Van Veelen, P.; Pena, S.; Mearin, L.; Papadopoulos, G.; Koning, F. *J. Immunol*., **1998**, *161*, 1585.
[4] Arentz-Hansen, H.; Korner, R.; Molberg, O.; Quarsten, H.; Vader, W.; Kooy, Y.M.C.; Lundin, K.E.A.; Koning, F.; Roepstorff, P.; Sollid, L.; McAdam, S.N. *J. Exp. Med*., **2000**, *191*, 603.
[5] Vader, L.; de Ru, A.; van der Wal, Y.; Kooy, Y.; Benckhuijsen, W.; Mearin, M.; Drijfhout, J.; van Veelen, P.; Koning, F. *J. Exp. Med*., **2002**, *195*, 643.
[6] Lundin, K.E.A.; Sott, H.; Hansen, T.; Paulsen, G.; Halstensen, T.S.; Fausa, O.; Thorsby, E.; Sollid, L. *J. Exp. Med.,* **1993**, 188, 187.
[7] van de Wal, Y.; Kooy, Y.; van Veelen, P.; Pena, S.; Mearin, L.; Molberg, O.; Lundin, K.; Sollid, L.; Mutis, T.; Benckhuijsen, W.; Drijfhout, J.; Koning, F. *Proc. Natl. Acad. Sci.USA,* **1998**, *95*, 10050.
[8] Arentz-Hansen, H.; McAdam, S.; Molberg, O.; Fleckenstein, B.; Lundin, K.; Jorgensen, T.; Jung, G.; Roepstorff, P.; Sollid, L. *Gastroenterology,* **2003**, *123*, 803.
[9] Molberg, O.; Kett, K.; Scott, H.; Thorsby, E.; Sollid, L.M.; Lundin, K.E.A. *Scand. J. Immunol*., **1997**, *46*, 103.
[10] Troncone, R.; Gianfrani, C.; Mazzarella, G.; Greco, L.; Guardiola, J.; Auricchio, S.; De Bernardinis, P. *Dig. Dis. Sci*., **1998**, *43*, 156.
[11] Troncone, R.; Franzese, A.; Mazzarella, G.; Paparo, F.; Auricchio, R.; Coto, I.; Mayer, M.; Greco, L. *Am. J. Gastroent*., **2003**, *98*, 590.
[12] Ventura, A.; Magazzu, G.; Greco, L. *Gastroenterology,* **1999**, *117*, 297.
[13] Holmes, G.K *Arch. Dis. Child*., **2002**, *87*, 495.
[14] Sollid, L.; Jabri, B. *Curr. Op. Immunol.,* **2005**, *17*, 595.
[15] Sategna-Guidetti, C.; Volta, U.; Ciacci, C.; Usai, P.; Carlino, A.; De Franceschi, L.; Camera, A.; Pelli, A.; Brossa, C.; *Am. J. Gastroent.,* **2001**, *96*, 751.
[16] Iltanen, S.; Collin, P.; Korpela, M.; Holm, K.; Partenen, J.; Polvi, A.; Maki, M. *Am. J. Gastroent.,* **1999**, *94*, 1042.
[17] Volta, U.; Rodrigo, L.; Granito, A.; Petrolini, N.; Muratori, P.; Muratori, L. *Am. J. Gastroent*., **2002**, *97*, 2609.
[18] Maki, M.; Mustalahti, K.; Kokkonen, J.; Kulmala, P.; Haapalahti, M.; Karttunen, T.; Ilonen, J.; Laurila, K.; Dahlbom, I.; Hansson, T.; Hopfl. P.; Knip, M. *N. Engl. J. Med.,* **2003**, *348*, 2517.
[19] Fasano, A.; Berti, I.; Gerarduzzi, T.; Not, T.; Colletti, RB.; Drago, S.; Elitsur, Y.; Green, P.H.; Guandalini, S.; Hill, I.D.; Pietzak, M.; Ventura, A.; Thorpe, M.; Kryszak, D.; Fornaroli, F.; Wasserman, S.S.; Murray, J.A.; Horvath, K. *Arch. Intern. Med.,* **2003**, *163*, 286.

[20] Margaritte-Jeannin, P.; Babron, M.C.; Bourgey, M.; Louka, A.S.; Clot, F.; Percopo, S.; Coto, I.; Hugot, J.P.; Ascher, H.; Sollid, L.M.; Greco, L.; Clerget-Darpoux, F. *Tissue Antigens,* **2004**, *63*, 562.
[21] Hernandez, L.; Green, P. *Curr. Gastroenterol. Rep*., **2006**, *8*, 383.
[22] Gianfrani, C.; Auricchio, S.; Troncone, R. *Immunol. Lett*., **2005**, *99*, 141.
[23] Maiuri, L.; Ciacci, C.; Auricchio, S.; Brown, V.; Quaratino, S.; Londei, M. *Gastroenterology,* **2000**, *119*, 996.
[24] Maiuri, L.; Ciacci, C.; Ricciarelli, I.; Vacca, L.; Raia, V.; Auricchio, S.; Picard, J.; Osman, M.; Quaratino, S.; Londei, M. *Lancet,* **2003**, *362*, 30.
[25] Mention, J.J.; Ahmed, M.B.; Begue, B.; Barbe, U.; Verkarre, V.; Asnafi, V.; Colombel, J.F.; Cugnenc, P.H.; Ruemmele, F.M.; McIntyre, E.; Brousse, N.; Collier, C.; Cerf-Bensussan, N. *Gastroenterology*, **2003**, *125*, 730.
[26] Hue, S.; Mention, J.J.; Monteiro, R.; Zang, S.L.; Collier, C.; Schmitz, J.; Verkarre, V.; Fodil, N.; Bahram, S.; Cerf-Bensussan, N.; Caillat-Zucman, S. *Immunity*, **2004**, *21*, 367.
[27] Meresse, B.; Chen, Z.; Ciszewski, C.; Tretiakova, M.; Bhagat, G.; Krausz, T.N.; Raulet, D.H.; Lanier, L.L.; Groh, V.; Spies, T.; Ebert, E.C.; Green, P.H.; Jabri, B. *Immunity,* **2004**, *21*, 357.
[28] Mowat, A. *Nat. Rev. Immunol*., **2003**, 3, 331.
[29] Battaglia, M.; Gianfrani, C.; Gregari, S.; Roncarolo, M.G. *Ann. N. Y. Acad. Sci.,* **2004**, *1029*, 142.
[30] Anderson, R.P.; van Heel, D.A.; Tye-Din, J.A.; Barnardo, M.; Salio, M.; Jewell, D.P.; Hill, A.V. *Gut,* **2005**, *54*, 1217.
[31] Barone, M.; Gimigliano, A.; Castoria, G.; Palella, G.; Maurano, F.; Paparo, F.; Maglio, M.; Nanayakkara, M.; Mineo, A.; Miele, E.; Troncone, R.; Auricchio, S. *Gut,* **2006**, *56*, 480.
[32] Daum, S.; Bauer, U.; Foss, H.D.; Schuppan, D.; Stein, H.; Riecen, E.O.; Ullrich, R. *Gut*, **1999**, *44*, 17.
[33] Wieser, H. *Acta Paediatr. Suppl*., **1996**, *412*, 3.
[34] Silano, M.; De Vincenzi, M. *Nahrung,* **1999**, *3*, 175.
[35] Auricchio, S.; De Ritis, G.; De Vincenti, M.; Mancini, E.; Minetti, M.; Sapora, O.; Silano, V. *Biochem. Biophys. Res. Commun*., **1984**, *121*, 428.
[36] Auricchio, S.; de Ritis, G.; De Vincenzi, M.; Occorsio, P.; Silano, V. *Paediat. Res*., **1982**, *16*, 1004.
[37] De Ritis, G.; Auricchio, S.; Jones, H.W.; Lew, E.; Bernardin, J.E.; Kasarda, D.D. *Gastroenterology,* **1988**, *94*, 41.
[38] Giovannini, C.; Sanchez, M.; Straface, E.; Scazzocchio, B.; Silano, M.; De Vincenti, M. *Toxicology,* **2000**, *145*, 63.
[39] Vader, W.; Kooy, Y.; Van Veelen, P.; De Ru, A.; Harris, D.; Benckhuijsen, W.; Pena, S.; Mearin, L.; Drijfhout, J.; Koning, F. *Gastroenterology,* **2002**, *122*, 1729.
[40] Tollefsen, S.; Arentz-Hansen, H.; Fleckenstein, B.; Molberg, O.; Raki, M.; Kwok, W.W.; Jung, G.; Lundin, K.E.A.; Sollid, L. J. *Clin. Invest.,* **2006**, *116*, 2226.
[41] Mamone, G.; Ferranti, P.; Melck, D.; Tafuro, F.; Longobardo, L.; Chianese, L.; Addeo, F. *FEBS Lett.,* **2004**, *562*, 177.
[42] Shan, L.; Molberg, O.; Parrot, I.; Hausch, F.; Filiz, F.; Gray, G.; Sollid, L.; Koshla, C. *Science,* **2002**, *297*, 2275.
[43] Gianfrani, C.; Troncone, R.; Magione, P.; Casentini, E.; De Pascale, M.; Faruolo, C.; Senger, S.; Terrazzano, G.; Southwood, S.; Auricchio, S.; Sette, A. *J. Immunol.,* **2003**, *170*, 2719.
[44] Maiuri, L.; Ciacci, C.; Raia, V.; Vacca, L.; Ricciarelli, I.; Raimondi, F.; Auricchio, S.; Quaratino, S.; Londei, M. *Gut,* **2001**, *48*, 418.
[45] Dieterich, W.; Ehnis, T.; Bauer, M.; Donner, P.; Volta, U.; Riecken, E.O.; Schuppan, D. *Nat. Med*., **1997**, *3*, 797.
[46] Aeschlimann, D.; Paulsson, M. *Thromb. Haemost*., **1994**, *71*, 402.
[47] Salmi, T.T.; Collin, P.; Jarvinen, O.; Haimila, K.; Partanen, J.; Laurila, K.; Korponay-Szabo, I.R.; Huhtala, H.; Reunala, T.; Maki, M.; Kaukinen, K. *Aliment. Pharmacol. Ther.,* **2006,** *24*, 541.
[48] Dieterich, W.; Trapp, D.; Esslinger, M.; Leidenberger, M.; Piper, J.; Hahn, E.; Schuppan, D. *Gut,* **2003**, *52*, 1562.
[49] Esposito, C.; Paparo, F.; Caputo, I.; Rossi, M.; Maglio, M.; Sblattero, D.; Not, T.; Porta, R.; Auricchio, S.; Marzari, R.; Troncone, R. *Gut,* **2002**, *51*, 177.
[50] Halttunen, T.; Mäki, M. *Gastroenterology,* **1999**, *116*, 566.
[51] Maki, M. *Lancet*, **1996**, *348*, 1046.
[52] Zanoni, G.; Navone, R.; Lunari, C.; Tridente, G.; Bason, C.; Sivori, S.; Beri, R.; Dolcino, M.; Valletta, E.; Corrocher, R.; Puccetti, A. *PLoS Med.,* **2006**, *3*, 1637.
[53] Granito A, Muratori P, Cassani F, Pappas G, Muratori L, Agostinelli D, Veronesi L, Bortolotti R, Petrolini N, Bianchi FB, Volta U. *Clin. Exp. Immunol*., **2004**, *137*, 386.
[54] Sanchez, D.; Tuckova, L.; Sebo, P.; Michalak, M.; Whelan, A.; Sterzl, I.; Jelinkova, L.; Havrdova, E.; Imramovska, M.; Benes, Z.; Krupickova, S.; Tlaskalova-Hogenova, H. *J. Autoimmunity,* **2000**, *15*, 441.
[55] Dicke, W.K.; Weijer, H.A.; van de Kamer, J.H. *Acta Paediatr*., **1953**, *42*, 34.
[56] Fasano, A.; Catassi, C. *Gastroenterology,* **2001**, *120*, 636.
[57] Catassi, C.; Fasano, A.; Corazza, G.R. The global village of celiac disease. Eds: *Italian Celiac Society, AIC Press* **2005**.
[58] van Heel, D.A.; Hunt, K.; Greco, L.; Wijmenga, C. *Best Pract. Res. Clin. Gastroenterol*., **2005**, *19*, 323.
[59] Margaritte-Jeannin, P.; Babron, M.C.; Bourgey, M.; Louka, A.S.; Clot, F.; Percopo, S.; Coto, I.; Hugot, J.P.; Ascher, H.; Sollid, L.M.; Greco, L.; Clerget-Darpoux, F. *Tissue Antigens*, **2004**, *63*, 562.
[60] Babron, M.C.; Nilsson, S.; Adamovic, S.; Naluai, A.T.; Wahlstrom, J.; Ascher, H.; Ciclitira, P.J.; Sollid, L.M.; Partanen, J.; Greco, L.; Clerget-Darpoux, F. *Eur. J. Hum. Genet.,* **2003**, *11*, 828.
[61] Vader, W.; Stepniak, D.; Kooy, Y.; Mearin, L.; Thompson, A.; van Rood, J.; Spaenij, L.; Koning, F. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*, 12390.
[62] Lopez-Vazquez, A.; Rodrigo, L.; Fuentes, D.; Riestra, S.; Bousono, C.; Garcia-Fernandez, S.; Martinez-Borra, J.; Gonzalez, S.; Lopez-Larrea. *Gut,* **2002**, *50*, 336.
[63] Papadopoulos, G.; Wijimenga, C.; Koning, F. *J. Clin. Invest*., **2001**, *108*, 1261.
[64] Monsuur, AJ.; de Bakker, P.I .; Alizadeh, B.Z.; Zhernakova, A.; Bevova, M.R.; Strengman, E.; Franke, L.; van't Slot, R.; van Belzen, M.J.; Lavrijsen, I.C.; Diosdado, B.; Daly, M.J.; Mulder, C.J.; Mearin, M.L.; Meijer, J.W.; Meijer, G.A.; van Oort, E.; Wapenaar, M.C.; Koeleman, B.P.; Wijmenga, C. *Nat. Genet.,* **2005**, *37*, 1341.
[65] Giordano, M.; Marano, C.; Mellai, M.; Limongelli, M.G.; Bolognesi, E.; Clerget-Darpoux, F.; Momigliano-Richiardi, P.; Greco, L. *Genes Immun.,* **2006,** *7*, 606.
[66] Sategna-Guidetti, C.; Pulitano, R.; Grosso, S; Ferfoglia, G. *J. Clin. Gastroenterol.*, **1993**, *17*, 123.
[67] Ziegler, A.G.; Schmid, S.; Huber, D.; Hummel, M.; Bonifacio, E. *JAMA,* **2003**, *290*, 1721.
[68] Hansen, D.; Brock-Jacobsen, B.; Lund, E.; Bjorn, C.; Hansen, L.P.; Nielsen, C.; Fenger, C.; Lillevang, S.T.; Husby, S. *Diabetes Care,* **2006**, *29*, 2452.
[69] Hummel, M.; Bonifacio, E.; Naserke, H.E.; Ziegler, A.G. *Diabetes Care,* **2002**, *25*, 1111.
[70] Maurano, F.; Mazzarella, G.; Luongo, D.; Stefanile, R.; D'Arienzo, R.; Rossi, M.; Auricchio, S.; Troncone, R. *Diabetologia,* **2005**, *48*, 931.
[71] Gianfrani, C.; Auricchio, S.; Troncone, R. *Expert. Opin. Ther. Targets*, **2006**, *10*, 601.
[72] Gianfrani, C.; Leving, M.; Sartirana, C.; Mazzarella, G.; Iaquinto, G.; Giardullo, N.; Zanzi, D.; Camarca, A.; Auricchio, S.; Troncone, R.; Roncarolo, M.G. *J. Immunol*., **2006,** *177*, 4178.
[73] Stepniak, D.; Spaenij-Dekking, L.; Mitea, C.; Moester, M.; de Ru, A.; Baak-Pablo, R.; van Veelen, P.; Edens, L.; Koning, F. *Am. J. Physiol. Gastrointest. Liver. Physiol.*, **2006,** *291*, G621.
[74] Gianfrani, C.; Siciliano, R.A.; Facchiano, A.M.; Camarca, A.; Mazzeo, M.F.; Costantini, S.; Salvati, V.M.; Maurano, F.; Mazzarella,G.; Iaquinto, G.; Bergamo, P.; Rossi, M . *Gastroenterology*, **2007**, *133*, 780.
[75] Fasano, A.; Not, T.; Wang, W.; Uzzau, S.; Berti, I.; Tommasini, A.; Goldblum, S.E. *Lancet,* **2000**, *355*, 1518.
[76] Larché, M.; Wraith, DC. *Nat. Med.*, **2005**, *11*, S69.
[77] Anderson, R.P.; Degano, P.; Godkin, A.J.; Jewell, D.P.; Hill, A.V.S. *Nat. Med*., **2000**, *6*, 337.

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