The immune recognition of gluten in coeliac disease

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Summary

Coeliac disease, the most common intestinal disorder of western populations, is an autoimmune enteropathy caused by an abnormal immune response to dietary gluten peptides that occurs in genetically susceptible individuals carrying the HLA-DQ2 or -DQ8 haplotype. Despite the recent progresses in understanding the molecular mechanisms of mucosal lesions, it remains unknown how increased amounts of gluten peptides can enter the intestinal mucosa to initiate the inflammatory cascade. Current knowledge indicates that different gluten peptides are involved in the disease process in a different manner, some fragments being 'toxic' and others 'immunogenic'. Those defined as 'toxic' are able to induce mucosal damage either when added in culture to duodenal endoscopic biopsy or when administered *in vivo*, while those defined as 'immunogenic' are able to specifically stimulate HLA-DQ2- or DQ8-restricted T cell clones isolated from jejunal mucosa or peripheral blood of coeliac patients. These peptides are able to trigger two immunological pathways: one is thought to be a rapid effect on the epithelium that involves the innate immune response and the other represents the adaptive immune response involving CD4^{+} T cells in the lamina propria that recognize gluten epitopes processed and presented by antigen presenting cells. These findings are the subject of the present review.

Keywords: coeliac disease, gluten, innate immunity, adaptive immunity

Definition and clinical description of coeliac disease

Coeliac disease (CD) may be considered the most common chronic inflammatory condition since the estimated prevalence in Western Countries is near to one per cent [1,2]. CD is an autoimmune enteropathy caused by an abnormal immune response to dietary gluten that occurs in genetically susceptible individuals [3]. The unique well established genetic factor is the HLA-DQ region at 6p21.3 [4] which, however, contributes no more than 40% of the risk, the non-HLA genes being the stronger determinant of CD susceptibility [5]. The broad spectrum of gluten-sensitive intestinal mucosal changes that are characteristic, albeit not pathognomonic of this condition, ranges from a complete disruption of the mucosal architecture with villous flattening and crypt hyperplasia, to a slight increase of inflammatory infiltrate in both the epithelium and lamina propria [6]. Furthermore, from *in vivo* and *ex vivo* challenges it has been shown that all the lesions comprise a dynamically interrelated series

of events that, however, do not necessarily occur in the same patient [7–9]. The wide spectrum of clinical manifestations comprises the classical features of intestinal malabsorption to atypical or asyntomatic cases that now are believed to represent the majority of patients [10]. The only treatment currently available is a lifelong strict adherence to a gluten-free diet that is followed by an amelioration or a normalization of the histological lesions [3,6].

As far as pathogenesis is concerned, CD represents a unique and privileged model since both an external trigger, the gluten peptides [11], and the autoantigen, the ubiquitous enzyme tissue transglutaminase (tTG) [12], have been identified. However, despite the great advances of the last decade in understanding the molecular mechanisms of mucosal lesions, our knowledge about the immune recognition of gluten and the consequent immune response is still far from being complete. Two pathways have been hypothesized to be triggered by these peptides: one is the direct effect on the epithelium that involves the innate immune response [13], the

other represents the adaptive immune response involving CD4+ T cells in the lamina propria that recognize processed gluten epitopes [14].

The external trigger

For the vast majority of human beings, cereals represent an important source of nutrients, whereas for CD patients certain cereal products represent poisons that not only destroy small intestinal mucosa [6], but also predispose to gastrointestinal malignancy [15,16]. These products constitute the storage protein fraction of the endosperm of the grains which, in the wheat, is classified into four classes depending on their solubility, the albumins being soluble in water, the globulins soluble in salt solution, the gliadins soluble in alcohol solution and the glutenins insoluble in neutral aqueous or saline solution and ethanol. Gluten, which is provided by both gliadins and glutenins, is the product of a ball of wheat flour dough that has been exhaustively washed in tap water, and the baking qualities of the wheat depends on its ability to trap carbon dioxide in dough. No nutritional value has been attributed to gluten. When collectively considered, the alcohol soluble fractions of cereals are designated as prolamins, a term reflecting the particular amino acid composition that is a high content of proline and glutamine and, depending on the cereal, they have been termed secalin for rye, hordein for barley, avenin for oats other than gliadin for wheat [17,18]. The gluten peptides and the related prolamins are responsible for triggering mucosal lesions in CD patients [18]. Among them, only gliadin has been investigated in great detail. Gliadin is an extremely heterogeneous mixture of proteins that contains at least 40 components which can be assigned, on the basis of their electrophoretic mobility at acidic pH, to four major groups (i.e. α-, β-, γ-, and ω-gliadins) [19] or, more modernly, into three major types according to their N-terminal amino acid sequence, designated as α-, γ- and ω-types [20,21]. Even if the correspondence between the old and the new nomenclature is not complete, it may be assumed that the electrophoretically separated α- and β-gliadins mainly constitute the α-type gliadins, while γ- and ω- gliadins are comprised into the γ- and ω-types, respectively [11].

Current understanding indicates that different gluten peptides are involved in the disease process in a different manner, some fragments being 'toxic' and others 'immunogenic'. Specifically, a fragment is defined 'toxic' if it is able to induce mucosal damage either when added in culture to duodenal mucosal biopsy [22], or when administered *in vivo* on proximal and distal intestine [6,23,24], whereas a fragment is defined 'immunogenic' if it is able to specifically stimulate HLA-DQ2- [25–28] or DQ8- [29–31] restricted T cell lines and T cell clones derived from jejunal mucosa or peripheral blood of coeliac patients. More in detail, as concerns 'toxicity', using *in vitro* jejunal biopsy organ culture, it was demonstrated that α-, β-, γ-, and ω-gliadins have a decreasing toxicity and the presence of similar N-terminal aminoacid sequence in rye and barley prolamins suggests that a certain amino acid sequence may constitute the toxic determinant [32,33]. After the full 266 amino acid sequencing of A-gliadin [34], a fraction of α-gliadin, a panel of gliadin peptides was synthesized and tested to evaluate their toxicity [22,35–40] as listed in Table 1, while only in the last few years, by combining reverse phase high performance liquid chromatography and mass spectrometry, a very high number of gliadin peptides have been purified and tested for their immunogenicity. The immunogenic peptides (or epitopes) are listed in Table 2 [25–31,41–46], and it is remarkable that some of them are immunodominant (those with activity designed as +++), which is the ability of such epitopes to elicit a strong T cell response. In addition, this term refers to another two factors, which may be considered as the consequences of the previous feature, that are: first, an immunodominant epitope elicits a specific T cell response from intestinal biopsies of nearly all patients, whereas those which are simply immunogenic stimulate T cells only in some patients, and second, if dietary gluten is administered to a patients who has been on a gluten-free diet, T cells responsive toward the immunodominant epitopes are detectable in peripheral blood significantly earlier than T cells that respond to the immunogenic epitopes [47]. Furthermore, some peptides identified as inactive or simply immunogenic in their native form become very potent stimulators of T cells after treatment with tTG (see below) [48], a process that can lead to epitope focusing during the course of the disease [49].

Table 1. Toxic gliadin peptides.

Amino acid sequences	Position	Toxicity	References
VPVPQLQPQNPSQQQPQEQ	α 3–21	−	[22,36,37]
PGQQQPFPPQQPY	α 31–43	+	[39]
PGQQQPFPPQQPYPQPQPF	α 31–49	+	[22,36,37]
PGQQQPFPPQQPYPQPQPFPSQQPY	α 31–55	+	[36,39]
PQPQPFPSQQPY	α 44–55	+	[39]
SQQPYLQLQPFPQPQLPY	α 51–70	+	[40]
LQLQPFPQPQLPYPQPQLPY	α 56–75	+	[57]
QQYPLGQGSFRPSQQNPQA	α 202–220	−	[37]
LGQGSFRPSQQN	α 206–217	+	[38]

Table 2. Immunogenic gliadin peptides.

Amino acid sequences	Position	Immunogenicity	References
VRVPVPQLQPQNPSQQQPQ	α-gliadin: 1–19	+	[41]
QNPSQQQPQEQVPLVQQQ	α-gliadin: 11–28	+	[41]
QVPLVQQQQFPGQQQPFPPQ	α-gliadin: 21–40	+	[41]
PGQQQPFPPQQPYPQPQPF	α-gliadin: 31–49	+	[41]
FPGQQQPFPPQQPYPQPQPF	α-gliadin: 30–49	+	[41]
QPYPQPQPFPSQQPYLQL	α-gliadin: 41–58	+	[41]
PQPFPSQQPYLQLQPFPQ	α-gliadin: 46–63	+	[42]
PQPQLPYPQPQLPY	α-gliadin: 62–75/(a)	+/++ +	[41,43]
QLQPFPQPQLPY	α-gliadin: 57–68 (a)	+/++ +	[25]
QLQPFPQ	α-gliadin: 57–63 (a)	+++	[26]
LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	α-gliadin: 57–89/(a)	+/++ +	[44]
QLQPFPQPQLPY	α-gliadin: 58–69/(a)	+/++ +	[44]
PQPQLPYPQPQLPY	α-gliadin: 63–76/(a)	+/+++	[44]
PFRPQQPYPQPQPQ	α-gliadin: 93–106 (a)	+	[45]
LIFCMDVVLQ	α-gliadin: 123–132	+	[46]
QQPLQQYPLGQGSFRPSQQNPQAQG	α-gliadin: 198–222	+	[27]
QYPLGQGSFRPSQQNPQA	α-gliadin: 203–220/(a)	+/+	[31]
PSGQGSFQPS	α-gliadin: 205–214	–	[27]
PSGQGSFQPSQQ	α-gliadin: 205–216/(a)	+/+++	[27,29]
SGQGSFQPSQQN	α-gliadin: 206–217/(a)	+/+++	[27,29]
QGSFQPSQQN	α-gliadin: 208–217/(a)	–/+++	[27,29]
LQPQQPFPQQPQQPYPQQPQ	γ-gliadin: 60–79	+	[43]
FPQQPQQPYPQQPQ	γ-gliadin: 66–78	+	[43]
FSQPQQQFPQPQ	γ-gliadin: 102–113/(a)	–/+	[43]
OQPQQSFPEQQ	γ-gliadin: 134–153/(a)	+/+++	[28]
VQGQGIIQPQQPAQL	γ-gliadin: 222–236/(a)	+/+	[45]
QQQQPPFSQQQQSPFSQQQQ	glutenin: 40–59/(a)	–/+	[45]
QQPPFSQQQQPLPQ	glutenin: 46–60/(a)	–/+	[45]
SGQGQRPGQWLQPGQGQQGYYPTSPQQSGQGQQLGQ	glutenin: 707–742/(a)	+/+	[30]
PGQGQQGYYPTSPQQSGQ	glutenin: 719–736	+	[30]
GYYPTSPQQSGQGQQLGQ	glutenin: 725–742	+	[30]
GYYPTSPQQSG	glutenin: 725–735	+	[30]
QGYYPTSPQQS	glutenin: 724–734/(a)	+ –	[30]
QQGYYPTSPQQSG	glutenin: 723–735	+	[30]
GQQGYYPTSPQQSG	glutenin: 722–735	+	[30]
GQQGYYPTSPQQS	glutenin: 722–734	+	[30]

(a): deamidated.

As mentioned, a common feature among gluten epitopes is the presence of multiple proline and glutamine residues, which make them exceptionally impregnable by gastric, pancreatic and intestinal digestive proteases [50]. In addition, the repetitive presence of these residues makes the peptides a preferred substrate of tTG, whose main function is to catalyse the covalent and irreversible crosslinking of a glutamine residue in glutamine-donor proteins with a lysine residue in glutamine-acceptor proteins which results in the formation of an ε-(γ-glutamyl)-lysine (isopeptidyl) bond [51]. However, apart from crosslinking its substrates, tTG can also hydrolyse peptide-bound glutamine to glutamic acid either at a lower pH or when no acceptor proteins are available [52], a process leading to an enhanced immunogenicity of gluten peptides [49]. Furthermore, these peptides naturally adopt a structural configuration characterized by a left-handed polyproline II helical confor-

mation, which is the one preferred by all bound HLA class II ligands [53].

Glutenins are also involved in T cell response, and specifically the native peptide HLA-DQ8-restricted glt04 707–742, whose minimal stimulatory sequence (residues 724–734) is repetitively present in the glutenin molecules [30], and the high molecular weight glutenin proteins in a HLA-DQ2-restricted and gliadin epitope-cross reactivity fashion [54]. It is remarkable that tTG specific deamidation does not influence the T cell recognition of the former peptide, whereas it is determinant in the latter.

More recently, using algorithms based on the spacing between glutamine and proline residues and on the presence or absence of other amino acids within the peptide, Vader *et al.* [32,55] predicted there are as many as 50 or more immunogenic peptides in gluten, hordeins, and secalins, but almost none in avenins. However, even though previous

studies have also found the absence of oats toxicity in both paediatric and adult CD patients [56–58], Sollid and co-workers [59] recently provided evidence of stimulatory capacity of such peptides overall after deamidation by tTG. They clearly demonstrated that even if some of the avenin-reactive T cells were originally primed to gluten and responded to avenin because of cross-reactivity, there is also a specific avenin-driven immune response.

Finally, even though discrepancies exist between toxicity and immunogenicity, since a peptide defined as toxic does not necessarily activate intestinal T cells from coeliac patients and, conversely, some of the immunostimulant peptides may not prove toxic to the intestinal mucosa of CD patients, it is not excluded that the same peptide may have both capacities simultaneously [40,60]. Further studies using a combination of epitope identification by T cell clones and epitope confirmation by *in vitro* biopsy challenge will identify a valuable map of the gliadin fragments and other gluten peptides involved in disease pathogenesis.

Gluten and the innate immune response

Although a number of studies have been published concerning the role of T cells in inducing intestinal damage [61–63], little is known about the early stages in which gluten starts the whole process. The primary event of a gluten-induced immune response requires epitope-bearing oligopeptides to have access to lamina propria inside the relatively impermeable surface of the intestinal epithelial layer. Ordinarily, such oligopeptides are efficiently hydrolysed into amino acids, di- or tri-peptides by peptidases located in the brush border membrane of the differentiated enterocytes before they can be transported across the epithelium. The importance of digestive processing for gluten antigenicity is supported not only by the observed proximal to distal gradient of the coeliac lesions in the small intestine, but mainly by the lack of an immune response following the prolyl endopeptidase digestion of the 33-mer immunodominat peptide [44]. An increased transport throughout the intestinal epithelium of both toxic and immunogenic gliadin fragments has recently been demonstrated only in active CD [64], pleading against a constitutive defect and suggesting that the immaturity of enterocytes and/or inflammation are responsible for this event. On the same line are the results previously obtained by Zimmer *et al.* [65] who demonstrated that gliadin is translocated into vacuoles positive for HLA class II antigens in enterocytes of untreated CD patients, but not in those of healthy controls. It is well known that under inflammatory and/or stress stimuli, enterocytes express not only HLA class II molecules, but also MHC class I chain-related gene (MIC) and HLA E molecules that are recognized by the natural killer receptors NKG2D and CD94 present on intraepithelial lymphocytes (IELs) [66,67]. The recent discoveries that interleukin (IL)-15, a key regulatory cytokine which supports the homeostasis between innate and adaptive immu-nity [68], may be produced by intestinal epithelial cells [69] and is a potent stimulant of IELs [70], and that gliadin causes an overexpression of MIC-A in the intestinal mucosa of CD patients [71] have focused the attention on IL-15 as a key molecule in the pathogenesis of CD [67,72–74]. Maiuri *et al.* [75] demonstrated that the toxic gliadin peptide 31–43 is able to induce an innate immune response by up-regulating the expression of IL-15, cyclo-oxygenase-2, and the activation markers CD25 and CD83 in lamina propria cells having the characteristics of macrophages, monocytes, and dendritic cells, without binding to HLA-DQ2 or -DQ8 and without stimulating CD4+ T cells. This study clearly shows that certain gluten peptides elicit an innate response, while others drive the adaptive immunity. Interestingly, the innate response to peptide 31–43 is only found in HLA-DQ2 positive CD patients, but not in HLA-DQ2 positive noncoeliac controls. This peculiar innate response to gluten may explain why only some of DQ-2 positive individuals develop CD, the prevalence of DQ2-positivity in western populations ranging between 25% and 35% [4]. Recently, another gluten peptide that activates the innate immune system has emerged [76], and the existence of more such fragments can be expected.

However, it remains unknown how very small amounts of gluten peptides can enter the intestinal mucosa of genetically susceptible subjects to initiate the inflammatory cascade. In rat intestine, gliadin administration leads to increased permeability of tight junctions, mediated by zonulin [77], and in human coeliac mucosa an increased zonulin expression has been demonstrated [78]. It can thus be hypothesized that in CD a gliadin-dependent or -independent breaking of the mucosal barrier facilitates the delivery of gluten peptides to the lamina propria via the paracellular route.

Another unsolved problem is how gluten peptides can cross the basement membrane to directly interact with immune cells present in the lamina propria. In this regard, intestinal epithelial cells have been shown to release exosome-like vesicles morphologically similar to those secreted by professional antigen presenting cells (APC) in fact, very recently, Van Niel *et al.* [79] clearly demonstrated that exosomes derived from intestinal epithelial cells are antigen presenting vesicles bearing HLA class II/peptide complexes and that they are up-regulated by interferon-γ treatment. Gluten fragments may therefore interact with the underlying immune system through exosomes released from the basolateral side of enterocytes. Once present in the lamina propria, peptides undergo capturing and processing by professional APC. Up to now, no direct information is available concerning the mechanisms of antigen processing and presentation of gluten peptides by subepithelial dendritic cells either in normal condition or in CD, even though an interesting paper has recently been published concerning the influence of digested wheat gluten on bone marrow-derived dendritic cells of BALB/c mice [80]. The authors demonstrated that gluten peptides are able to induce both maturation and expression of costimulatory molecules by dendritic
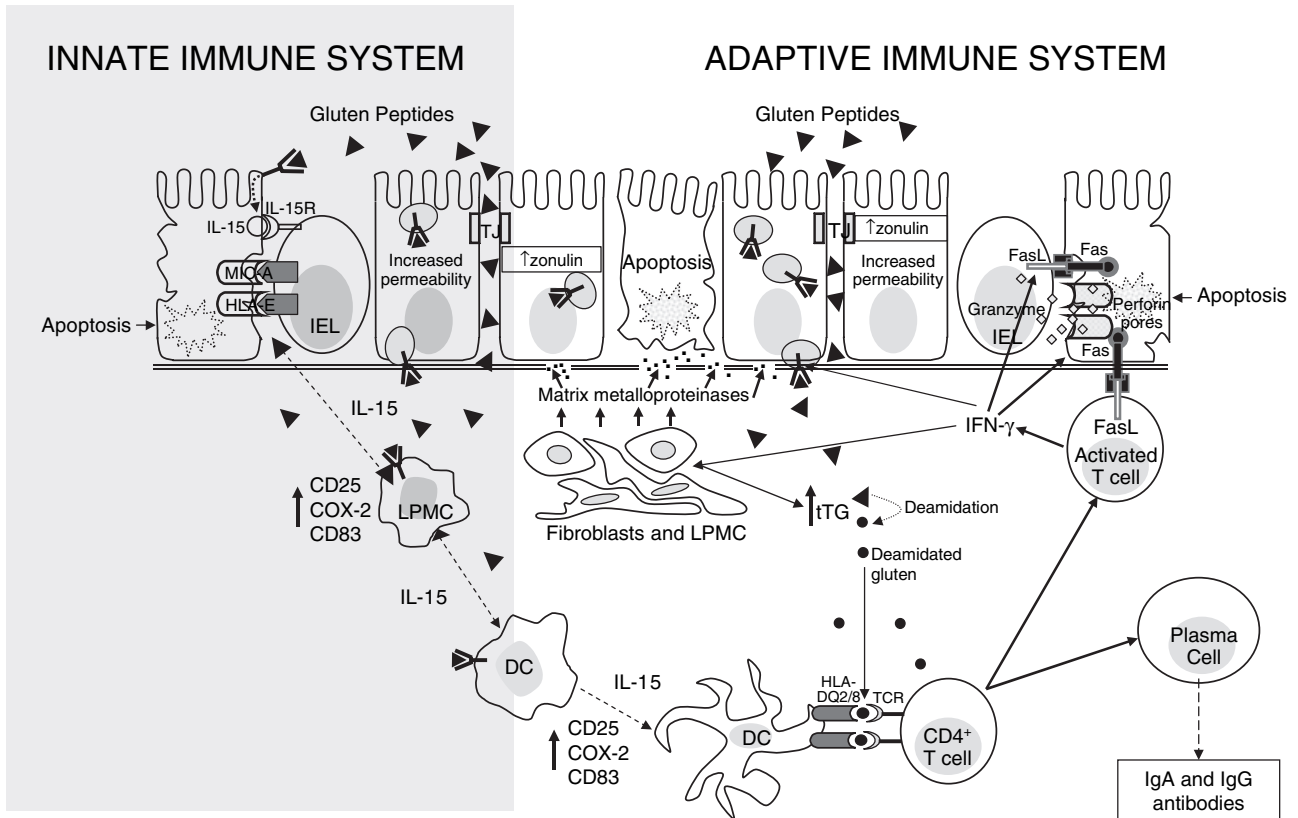
Fig. 1. Epithelial transport and immune recognition of gluten in coeliac disease. In coeliac disease gluten peptides may cross the intestinal epithelium either via the paracellular route or via the transcellular route. In the first case this occurs as a consequence of an increased permeability due to an up-regulation of zonulin (which might be gliadin-dependent or gliadin-independent), in the second case it occurs by using enterocytic vesicles carrying the HLA class II-peptides complex. These vesicles are able to cross the basal membrane allowing intact gluten peptides to have access to the lamina propria. Here, they can activate both dendritic cells and lamina propria mononuclear cells to produce interleukin 15 which in turn causes an up-regulation of stress protein by enterocytes that are recognized by the natural killer receptors present on intraepithelial lymphocytes. On the other hand, native and tTG-deamidated gluten peptides are presented by mature dendritic cells to T cell receptors by using the heterodimer encoded by HLA-DQ2 or DQ8 genes A and B. This bond strongly activates CD4^{+} T cells which, by secreting pro-inflammatory cytokines, stimulate cytotoxic T cells and fibroblasts to produce a particular matrix metalloproteinase pattern which is responsible for degradation of both extracellular matrix and basement membrane. Activated T cells also become able to trigger enterocyte apoptosis by producing molecules like Fas ligand and granzyme, which are responsible for cytotoxicity, leading to the characteristic mucosal lesions. Stimulated CD4^{+} T cells are also able to induce lymphocyte B differentiation into plasma cells producing specific antigladin and anti-tissue transglutaminase antibodies. DC, dendritic cell; FasL, Fas ligand; HLA, human leucocyte antigen; IL, interleukin; IEL, intraepithelial lymphocyte; LPMC, lamina propria mononuclear cell; TCR, T cell receptor; TJ, tight junction; tTG, tissue transglutaminase.

cells, and the secretion of chemokines responsible for leucocyte activation.

The described alterations in transport and processing of gluten peptides by intestinal mucosa of CD patients likely participate in the pathogenesis of the disease and the combined presence of toxic and immunogenic sequences may stimulate synergistically both the innate and adapive immune system (Fig. 1).

Gluten and the adaptive immune response

The presence of an increased amount of gluten peptides in the lamina propria together with a genetic predisposition greatly increases the risk of breaking the oral tolerance toward these peptides. As concerns the genetic predisposi-

tion, a condition necessary but not sufficient for the development of the disease process, two highly polymorphic genes called DQA1 and DQB1, encoding the α/β transmembrane heterodimer expressed on the surface of APC [81] have been well characterized. The vast majority of patients, approximately 95%, have the DQ2 haplotype (DQA1*0501/DQB1*0201, termed HLA-DQ2.5 hereafter) and most of them are DR3-DQ2 homozygotes (DRB1*0301-DQA1*0501-DQB1*0201) or are DR5-DQ7 and DR7-DQ2 heterozygotes (that is DRB1*11/12-DQA1*0505-DQB1*0301 and DRB1*07-DQA1*0201-DQB1*0202, respectively) [82]. A second HLA-DQ2 molecule exists (DQA1*0201/DQB1*0202, termed HLA-DQ2.2 hereafter) with peptide-binding properties that are virtually identical to those of HLA-DQ2.5 [83]. However, HLA-DQ2.2 does not

predispose for CD unless it is expressed together with HLA-DQ2.5 [84]. Specifically, individuals homozygous for HLA-DQ2.5 genotype or HLA-DQ2.2/2.5 heterozygous have a higher risk of developing CD, while those HLA-DQ2.5/non DQ2.2 heterozygous have only a slightly increased risk [82]. These findings have recently been confirmed by an *in vitro* study aimed at evaluating the ability of gluten peptides to stimulate T cell clones in the presence of an HLA-DQ2 positive EBV-transformed lymphoblastoid cell line used as APC [84]. The authors concluded that the risk associated with the HLA-DQ2 haplotype depends on the impact of the number and quality of the HLA-DQ molecules on gluten peptide presentation to T cells. The remainder of DQ2 negative coeliac patients (about 5%) mostly have a DQ8 molecule encoded by DQB1*0302 and DQA1*0301 on a DR4 haplotype [85].

In CD, the HLA-DQ2 and -DQ8 molecules bind protein fragments in their peptide grooves, and these complexes are expressed on the cellular surface of APC to be recognized by a specific population of CD4+ T cells. A very important finding is that optimal binding of some gluten peptides to HLA-DQ2/DQ8 requires their prior modification by tTG [86,87]. As previously mentioned [49,52], this enzyme has a high avidity for prolamins and possesses, at low pH, a deamidation activity which transforms neutral glutamine residues into negatively charged glutamic acid [88]. The introduction of negative charges into gluten peptides at specific positions favours their interaction with basic amino acids located in the anchor positions of HLA-DQ2/DQ8 molecules, and subsequently enhances peptide binding and T cell stimulation [86,87]. Whether immunogenic gluten peptides are recognized by mucosal T cells in an isolated form and/or in a complexed form, since the presence of gluten-tTG complexes has recently been demonstrated in coeliac mucosa [89], remains to be established.

It is of interest to note that the expression levels of HLA-DQ are under the influence of cytokines and particularly of interferon-γ [90]. Local production of interferon-γ, e.g. as the result of an infection, would thus lead to elevated HLA-DQ expression by professional and nonprofessional APC like enterocytes and, consequently, gluten peptide presentation, which can contribute to the development and/or progression of a gluten-specific T cell response. Furthermore, the X-ray crystal structure of the soluble domain of HLA-DQ2 bound to a deamidated epitope of αI-gliadin has recently been reported, and demonstrates the presence of an intricate hydrogen-bonding network between the two molecules [53]; it was demonstrated that the deamidated αI-gliadin has a 25-fold higher affinity compared with the nondeamidated counterpart probably due to having a more potent hydrogen-bond acceptor that can form additional protein-to-protein hydrogen bonds, resulting in a more stable gliadin-DQ2 complex.

This properly presented epitope engages the T cell receptor on CD4+ T lymphocytes which then become able to activate cytotoxic T lymphocytes, macrophages, stromal cells and B lymphocytes by secreting a peculiar pattern of cytokines and matrix metalloproteinase [91], which in turn cause tissue damage and antibody production (Fig. 1).

Conclusions

It is hoped that the knowledge of the exact nature of the gluten peptides and of the mechanisms of their intestinal handling in CD patients will make it feasible to inhibit or prevent the disease by blocking the different steps of gluten recognition. It has been demonstrated that the substitution of a glutamine residue by a proline residue in both glutenin and gliadin epitopes lacked T cell recognition and then immunostimulatory activity [32], and it is also demonstrated that by a single amino acid substitution, the 31–49 peptide looses its toxic property [92]. Theoretically, the specific substitution at DNA level may allow the detoxification of gluten peptides without affecting the baking properties, but this procedure have some intrinsic problems since several nucleotide mutations might be necessary to eliminate T cell reactivity, gluten peptides are encoded by many different genes on distinct chromosomes and the final viability of such genetically modified wheat is unknown. Further difficulties are due to the presence of different epitopes by age, children being sensitive to an enormous number of epitopes in contrast to adult patients [45,49], and to the possibility that the removal of known epitopes could reveal alternative epitopes like what happens for avenin [59]. The alternative might be to detoxify gluten by the action of bacterial prolyl endopeptidase [44], such as vaccinotherapy by intranasal administration of whole gliadin or its epitopes able to induce systemic T-cell anergy [93,94], or the use of analogue peptides able to bind HLA class II molecules [53]. The future availability of an animal model of CD may make it possible to evaluate all the new therapeutic strategies for this pathological condition [95,96].

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