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## **Celiac disease : from basic insight to therapy development**

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# 1

## General introduction: Celiac disease - sandwiched between innate and adaptive immunity

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# General Introduction:

## Celiac Disease - Sandwiched Between Innate and Adaptive Immunity

**Dariusz Stepniak and Frits Koning**

### **ABSTRACT**

Celiac disease (CD) patients are intolerant to gluten, proteins in wheat and related cereals. Virtually all patients are HLA-DQ2 or HLA-DQ8 positive and several studies have demonstrated that CD4 T cells specific for (modified) gluten peptides bound to these HLA-DQ-molecules are found in patients but not in control subjects. These T cell responses are therefore thought to be responsible for disease development. Many immunogenic gluten peptides have now been identified which may relate to the disease inducing properties of gluten. In addition, gluten can stimulate IL-15 production that ultimately leads to NKG2D-mediated epithelial cell killing. However, CD develops in only a minority of HLA-DQ2 and HLA-DQ8 individuals. This may be attributed to the default setting of the intestinal immune system: induction and maintenance of tolerance to dietary components and commensal flora. Although it is at present unknown why in CD tolerance is not established or broken, both environmental and genetic factors have been implicated. There is strong evidence for the existence of genes or gene variants on chromosome 5, 6 and 19 that predispose to CD. In addition, type I interferons have been implicated in development of several autoimmune disorders, including CD. Thus, viral infection and/or tissue damage in the intestine may cause inflammation and induce protective Th1-mediated immunity leading to loss of tolerance for gluten. Once tolerance is broken, a broad gluten reactive T cell repertoire may develop through determinant spreading. This may be a critical step towards full-blown disease.

## **Introduction**

The development of agriculture, which started in the Middle East about 10,000 years ago, not only led to the development of ancient civilizations but also resulted in radical changes in the composition of the human diet. One of those changes was the introduction of cereal based food products and today such food products are very common in a normal diet. Yet approximately 1% of the population in the Western world cannot tolerate cereals and suffers from celiac disease. Celiac disease (CD) is most likely as old as cereal consumption and its symptoms were described already by the Roman physician Galen. But it was not until the 1950's that gluten, the grain storage proteins, was found to be responsible for the occurrence of the clinical manifestations in CD patients. More recently, the role of HLA in the development of an inflammatory T cell response to the gluten has been elucidated. It is still unclear, however, why only a minority of predisposed individuals actually develop CD. Here we describe a number of recent observations that shed light on the fatal interaction between gluten and the immune system and discuss their implications.

## **Presentation**

CD is a small intestinal disorder and common manifestations include chronic diarrhea, abdominal distension and malnutrition. These symptoms result from an inflammatory immune response to wheat gluten and related proteins in barley and rye causing villous atrophy, hypertrophic crypts and infiltration of intraepithelial lymphocytes (IELs) in the small intestine. The clinical picture normalizes upon strict compliance to a gluten-free diet. CD can occur early in life, short after the introduction of gluten into the diet, but it can also develop much later in life. Also, the disease severity varies significantly between patients and only a minority present with the typical symptoms associated with CD. Although several screening studies have indicated that the prevalence of CD in the western populations is between 0.5-2%, only about 10% of these are diagnosed and follow a gluten-free diet [1].

In some patients the activation of intraepithelial lymphocytes cannot be controlled by a gluten free diet. This so-called refractory CD disease can lead to the development of T cell lymphoma [2].

## **Gluten and HLA-DQ**

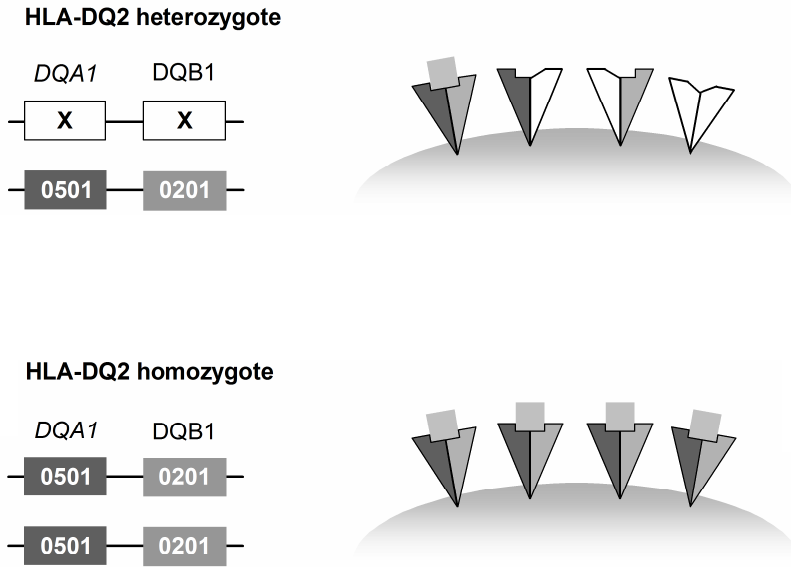
Celiac disease is a multifactorial disorder in which both genetic and environmental factors contribute to disease development. The concordance rate in monozygotic twins is 86% whereas in dizygotic twins it reaches only 20%, pointing to a strong impact of genetic factors [3]. Of these HLA is estimated to be responsible for

|                              |          |   |   |          |          |          |          |   |          |
|------------------------------|----------|---|---|----------|----------|----------|----------|---|----------|
| Glia- $\alpha$ 2 (Alpha-II)  | <b>P</b> | Q | P | <u>Q</u> | L        | <b>P</b> | <b>Y</b> | P | <b>Q</b> |
| Glia- $\alpha$ 9 (Alpha-I)   | <b>P</b> | F | P | <u>Q</u> | P        | <u>Q</u> | <b>L</b> | P | <b>Y</b> |
| Glia- $\alpha$ 9 (Alpha-III) | <b>P</b> | Y | P | <u>Q</u> | P        | <u>Q</u> | <b>L</b> | P | <b>Y</b> |
| Glia- $\alpha$ 20            | <b>F</b> | R | P | <u>Q</u> | Q        | <b>P</b> | <b>Y</b> | P | <b>Q</b> |
| Glu-5                        | <b>Q</b> | X | P | <u>Q</u> | Q        | <b>P</b> | <u>Q</u> | Q | <b>F</b> |
| Glia- $\gamma$ 1 (Gamma-I)   | <b>P</b> | Q | Q | <b>S</b> | F        | <b>P</b> | <u>Q</u> | Q | <u>Q</u> |
| Glia- $\gamma$ 2             | <b>P</b> | F | P | <u>Q</u> | Q        | <b>P</b> | <u>Q</u> | Q | <b>P</b> |
| Glia- $\gamma$ 30 (Gamma-II) | <b>I</b> | I | Q | <b>P</b> | <u>Q</u> | <b>Q</b> | <b>P</b> | A | <b>Q</b> |
| Gamma-III                    | <u>Q</u> | Q | P | <u>Q</u> | Q        | <b>P</b> | <b>Y</b> | P | <b>Q</b> |
| Gamma-IV                     | <b>S</b> | Q | P | <u>Q</u> | Q        | <b>Q</b> | <b>F</b> | P | <b>Q</b> |
| Glt-156                      | <b>P</b> | F | S | <u>Q</u> | <u>Q</u> | <b>Q</b> | <u>Q</u> | S | <b>P</b> |
| Glt-17 <sup>A</sup>          | <b>P</b> | F | S | <u>Q</u> | <u>Q</u> | <b>Q</b> | <u>Q</u> | Q | <b>P</b> |
| Glt-17 <sup>B</sup>          | <b>P</b> | F | S | <u>Q</u> | <u>Q</u> | <b>Q</b> | <u>Q</u> | P | <b>V</b> |
|                              | <b>F</b> |   |   | <b>E</b> |          | <b>P</b> | <b>E</b> |   | <b>F</b> |
|                              | <b>Y</b> |   |   | <b>D</b> |          | <b>E</b> | <b>D</b> |   | <b>Y</b> |
| Binding                      | <b>W</b> |   |   | <b>L</b> |          | <b>A</b> |          |   | <b>W</b> |
| motif                        | <b>L</b> |   |   | <b>I</b> |          |          |          |   | <b>L</b> |
|                              | <b>I</b> |   |   | <b>V</b> |          |          |          |   | <b>I</b> |
|                              | <b>V</b> |   |   |          |          |          |          |   | <b>V</b> |
|                              | <b>M</b> |   |   |          |          |          |          |   | <b>M</b> |

**Figure 1.** The HLA-DQ2 binding motif and the alignment of selected T cell stimulatory gluten peptides. The deamidated glutamine residues are underlined. Anchors are given in bold.

40-50% of the genetic contribution in CD [4,5]. While roughly 95% of patients carry HLA-DQ2 (DQA1\*0501/DQB1\*0201), most individuals that are not HLA-DQ2 positive express HLA-DQ8 (DQA1\*0301/DQB1\*0302). Both HLA-DQ2 and HLA-DQ8 have very characteristic peptide binding motifs characterized by a preference for hydrophobic and negatively charged amino acids at specific positions in bound peptides [6].

Gluten is a complex mixture of wheat storage proteins called gliadins and glutenins. Analogous proteins are present in rye, barley and oats and the detrimental effects of their consumption in celiac patients are well documented. Gluten proteins have several unique features that contribute to their immunogenic properties. They are extremely rich in the amino acids proline and glutamine. Due to the high proline content gluten is highly resistant to proteolytic degradation within the gastrointestinal tract as gastric and pancreatic enzymes lack post-proline cleaving activity. Moreover, the high glutamine content makes gluten a good substrate for the enzyme tissue transglutaminase (tTG). This enzyme is constitutively expressed in the lamina propria and released upon tissue damage. It is known to



**Figure 2.** The gluten binding capacity of APCs homozygous or heterozygous for HLA-DQ2. Transdimer formation in HLA-DQ2 heterozygots results in significantly decreased gluten presentation.

play a role in tissue repair but it can also convert glutamine into the negatively charged glutamic acid, a process called deamidation. Such modified gluten peptides can bind to HLA-DQ2 or HLA-DQ8 as these molecules have a preference for peptides with negatively charged amino acids at multiple anchor positions (Fig. 1). Deamidation is most likely a crucial event in the generation of a full-blown gluten-specific T cell response and concomitant disease development. Finally, gluten is now known to encode many peptides with T cell stimulatory capacity. While some of those peptides seem to be immunodominant as they evoke T cell responses in the large majority of patients, others appear to be less immunogenic. Nevertheless, once a T cell response to a particular gluten peptide has been initiated, a broad gluten-specific T cell response develops: T cells specific for multiple gluten peptides are found in virtually all patients. The generation of such a broad T cell response may be a prerequisite for disease development.

### HLA-DQ2 gene dose effect

Already in the 1980's it was observed that HLA-DQ2 homozygous individuals have an at least 5-fold higher risk of disease development compared to HLA-DQ2 hetero-

zygous individuals [7]. Recently, we demonstrated that this correlates with the strength of the gluten-specific T cell response: antigen presenting cells (APCs) homozygous for HLA-DQ2 elicit much stronger gluten-specific T cell responses compared to APC heterozygous for HLA-DQ2 [8]. This can be explained by the formation of HLA-DQ-transdimers on APCs heterozygous for HLA-DQ2, resulting in a much lower abundance of HLA-DQ2 molecules capable of presenting gluten peptides on such APC (Fig. 2). Most likely the same holds true for HLA-DQ8. These observations imply that the quantity of HLA-DQ2, and thus presumably the quantity of cell surface HLA-DQ2-gluten peptide complexes, co-determines the likelihood of disease development. Conversely, this might indicate that lowering the exposure to gluten could be a very effective way of reducing the incidence of CD. In this respect it is important to note that the introduction of relatively large amounts of gluten to infants' diet after in the mid 1980's in Sweden caused a dramatic increase in the incidence of symptomatic CD in children younger than 2 years [9].

Interestingly, many of the refractory CD patients are homozygous for HLA-DQ2 [10], suggesting that the high intensity of the specific anti-gluten responses contributes to an uncontrolled non-specific inflammatory reaction that can ultimately lead to lymphoma.

### **Oral tolerance**

The gut-associated lymphoid tissue (GALT) is the largest and probably most complex part of the immune system. It is in continuous contact with a complex mixture of foreign antigens over a surface measuring 400 square meters. The GALT has to discriminate between pathogenic microorganisms and harmless antigens such as dietary compounds and commensal bacteria. The default set-up of the intestinal immunity is therefore the generation of tolerance, unless specific signals evoke inflammatory reactions. The antigens present in the gut lumen are constantly sampled by intestinal dendritic cells and presented to the T cells in either Peyer's patches or mesenteric lymph nodes, which results in the generation of regulatory CD4<sup>+</sup> T cells [11]. Moreover, high concentrations of anti-inflammatory cytokines such as IL-10 and TGF- $\beta$  are normally found in the intestine. Therefore it seems unlikely that gluten could initiate adaptive immune responses by itself. Rather, inflammatory stimuli are required to polarize the normally quiescent and tolerogenic dendritic cells so that they will generate Th1 responses.

### **How is tolerance broken?**

Several mechanisms have been proposed that could lead to the development of a gluten specific T cell response. First, cross-reactivity between autoantigens and pathogen-derived antigens can lead to the development of autoimmunity. Similarly, it has been hypothesized that molecular mimicry between pathogens and gluten

could trigger the immune responses that result in CD. Already in 1984 it was observed that the E1B protein of human adenovirus type 12 shares sequence homology with an  $\alpha$ -gliadin fragment [12]. However, no viral DNA nor antibodies specific for the E1B protein were found in patients [13-15]. Also, patient-derived T cells that are specific for the  $\alpha$ -gliadin peptide do not cross-react with the homologous viral peptide (unpublished results). Therefore, at present no direct evidence supports the implication of adenoviral infections in disease development.

More recently the hyphal wall protein 1 (HWP1) from *Candida albicans* was shown to share sequence homology with  $\alpha$ - and  $\gamma$ -gliadins [16]. Based on this a direct role for Candida specific T cell responses in triggering the onset of CD was hypothesized but again this has not been confirmed by experimental findings and remains speculative.

Similarly, a role for bacteria in pathogenesis of CD was postulated due to the finding that rod-shaped bacteria were frequently associated with the mucosa in CD patients, with both active and inactive disease, but not in controls [17]. This difference in the colonization pattern between CD patients and healthy controls could result from differences in glycocalyx composition, but a direct role for these bacteria in disease development has not been shown.

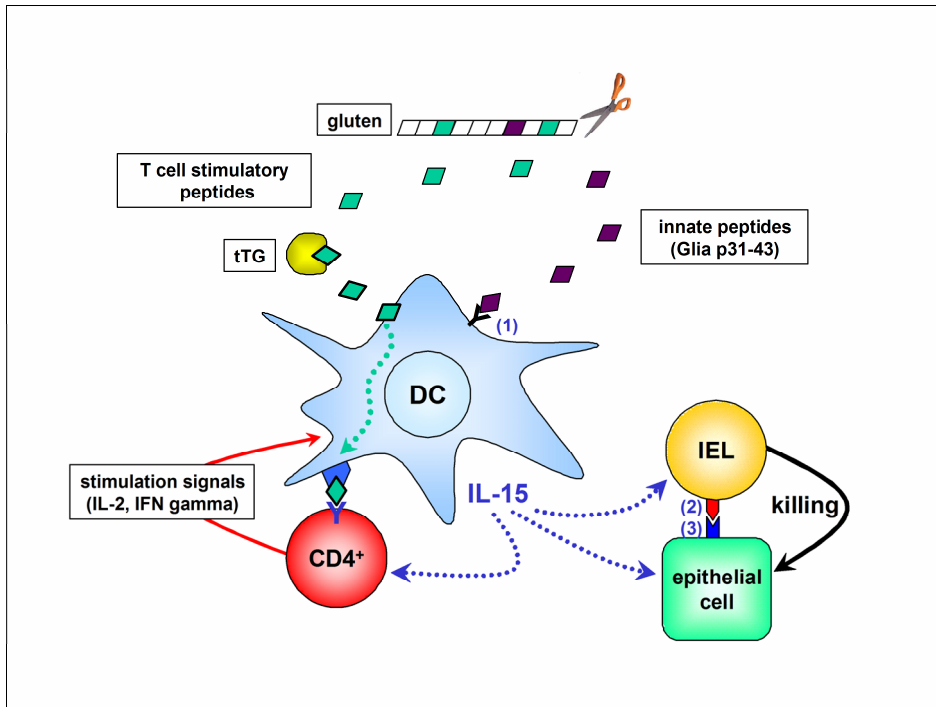
### **Type I interferons and viruses**

There is, however, a growing body of evidence suggesting a role for interferon-alpha (IFN- $\alpha$ ) in CD development. In two cases the onset of CD was observed during treatment with IFN- $\alpha$  for chronic hepatitis C and chronic myeloid leukemia [18-20]. A potential role for IFN- $\alpha$  is further supported by the observation that T cell activation with anti-CD3 antibody in explant cultures of human fetal gut results in villous atrophy and crypt cell hyperplasia only in the presence of IFN- $\alpha$  [21].

Type I IFNs exert potent antiviral and immune-regulating activity promoting the differentiation and maintenance of Th1 cells [22]. They can be secreted in the response to infection with viruses and intracellular bacteria and enteroviral infections can provoke the secretion of IFN- $\alpha$  in the intestine [23], and this results in an upregulation of IFN- $\gamma$  and IL-15 production by DCs [24,25], two proinflammatory cytokines that are known to be involved in the pathogenesis of CD [26,27].

The role of interferons in autoimmune diseases such as systemic lupus erythematosus (SLE), type I diabetes (T1D) and rheumatoid arthritis (RA) is well documented. Increased serum levels of IFN- $\alpha$  in SLE patients have been measured and they clearly correlate with the disease exacerbation [28]. In T1D patients increased plasma levels of IFN- $\alpha$  were found to be associated with Coxsackie B virus infections [29] and, similar to CD, IFN treatment can cause onset of T1D, RA, myasthenia gravis and autoimmune hemolytic anemia [30-34]. Moreover, in mice





**Figure 3.** Gluten-specific T cells in the lamina propria proliferate and produce proinflammatory cytokines such as IL-2 and IFN- $\gamma$ . Stimulated DCs express a hypothetical gliadin receptor (1), which upon triggering with innate immunity stimulatory fragments (e.g. Glia p31-43) further stimulates DCs and provokes IL-15 production. IL-15 stimulates IELs to express NKG2D receptors (2) and epithelial cells to express MICA molecules (3). Upon engagement of NKG2D receptor with MICA ligand the IELs kill the epithelial cells causing the tissue destruction. The identity of the putative Glia p31-43 receptor remains unknown.

transgenic expression of IFN- $\alpha$  or IFN- $\beta$  in pancreatic  $\beta$ -cells results in overt diabetes [35,36].

Virtually every child experiences episodes of rotavirus gastroenteritis in the first two years after birth [37,38]. Also other enteroviruses including astroviruses, noroviruses and adenoviruses are a frequent cause of diarrhea episodes in children [38]. Th1 responses and inflammation play an important role in the anti-enteroviral immunity [39,40] and are associated with local production of IFN- $\alpha$ . [41,42]. It is therefore conceivable that IFN- $\alpha$  production as a result of a viral infection would lead to a shift towards Th1 responses and reinstruct previously tolerogenic DCs to prime gluten-specific T cells and support inflammation instead of sustaining oral tolerance. The subsequent cytokine production, in particular IFN- $\gamma$ , would cause an upregulation of HLA-expression facilitating T cell priming, expansion and deter-

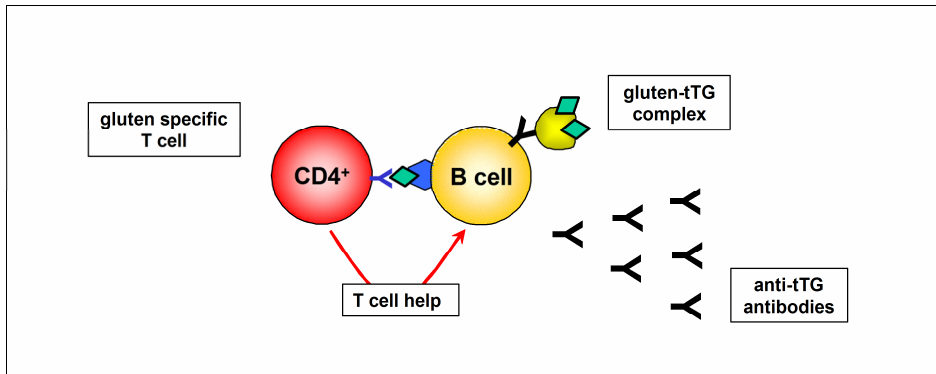
minant spreading [43]. T cell cross-reactivity towards various gluten epitopes and homologous peptides in other cereals may further contribute to the spreading of the T cell responses [44]. Ultimately this results in full-blown disease.

### **Cross-talk between innate and adaptive immunity**

The induction of an adaptive immune response is tightly controlled by innate immunity. Dendritic cells, the sentinels of the immune system, not only recognize invading pathogens but also decide what type of effector responses should be deployed. It is clear that without signals provided by intestinal DCs no gluten-specific T cell responses could develop. Quite recently it has been demonstrated that gliadin is capable of stimulating cytokine production by human macrophage line THP-1 [45] and inducing the maturation of monocyte-derived dendritic cells [46]. Studies using *ex vivo* tissue culture models showed that gliadin, and the gliadin derived fragment p31-43, can induce IL-15 secretion which results in upregulation of NKG2D on intraepithelial lymphocytes (IEL) and the NKG2D ligand MICA on epithelial cells [47]. *In vitro*, this has been shown to result in target cell killing [48,49]. An increase in the number of IEL in the small-intestinal biopsies of CD patients was noticed already a long time ago but the role of these cells in the pathogenesis of CD remained unclear until recently. These new results now indicate that IL-15 secretion can lead to epithelial cell destruction by IEL *in vivo*, a process that could contribute to the disappearance of the villi and flattening of the intestinal epithelium that is so characteristic for CD. IL-15 is most likely produced by activated intestinal dendritic cells and possibly other antigen presenting cells. In this scenario, the dendritic cells simultaneously induce two effector immune responses: adaptive – a gluten-specific CD4<sup>+</sup> T cell response, and innate – mediated by IEL (Fig. 3). The production of IL-15 by dendritic cells would thus partly depend of the gluten specific T cell response and this might explain why the induction of an innate response by gliadin is only observed in biopsies from CD patients and not healthy controls. A proinflammatory status of the tissue may thus be a prerequisite for the innate immunity stimulation by gliadin *in vivo*. The mechanism by which gliadin, and in particular the gliadin fragment p31-43, can directly stimulate IL-15 production remains unknown but a recent study suggested a role for tTG in this process [50].

### **Autoantibodies to tissue transglutaminase are specific indicators of CD**

The presence of serum autoantibodies directed against tissue transglutaminase is a specific marker for active celiac disease. The mechanism of their formation has not been fully explained. Since no tTG-specific T cells providing help to the antibody-producing B cells have been found it has been proposed that the necessary help comes from gluten-reactive T cells. This hypothesis is supported by the observation that tTG can cross-link itself to gluten molecules. Such complexes could be taken up



**Figure 4.** The hypothetical model explaining antibody production in celiac patients. Tissue transglutaminase specific B cells endocytose gluten-tTG complexes and present gluten peptides to the gluten-reactive T cells. The stimulated T cells pay back with help for antibody production.

by tTG-specific B cells and, after intracellular degradation, gluten-derived would be presented to gluten-specific T cells in context of HLA-DQ2 or DQ8. These T cells, in turn, would provide the necessary help for antibody production (Fig. 4). This scenario fits with the observation that when patients are treated with a gluten-free diet the titers of the anti-tTG antibodies decrease. Since in the absence of gluten the gluten-reactive T cells are not stimulated, they cannot provide help to the B cells and antibody production stops [51]. Thus, although the antibodies are very specific indicator of CD, their formation is driven by the gluten specific T cell response. Moreover, the anti-tTG antibodies do not seem to contribute to the formation of the intestinal lesions in CD as disease symptoms disappear rapidly after the introduction of a gluten-free diet while antibody titers drop much slower. Similarly, in type I diabetes autoantibodies against islet-cells, glutamic acid decarboxylase (GAD), protein-tyrosine phosphatase-2 and insulin can be found, which might reflect the ongoing autoimmune process but most likely do not participate in the tissue damage [52].

### Other genetic factors

Next to HLA-DQ several other genetic factors are thought to contribute to the risk of disease development. A very recent breakthrough has been the identification of the first non-MHC coded gene associated with an increased risk for CD: myosin IXB (MYO9B) [4]. Although the mechanism by which this gene predisposes to CD has not been established, it has been speculated that this unconventional myosin molecule could account for an affected integrity of the intestinal barrier, a possibi-

lity that has been suggested in previous studies but has not been demonstrated [53]. Clearly, a "leaky gut" would allow an increase in the penetration of gluten peptides through the intestinal epithelium, enhanced deamidation of these peptides by tTG, and thus could contribute to an increased risk of breaking oral tolerance to gluten proteins.

MYO9B, however, is one of most likely many genes that are involved in CD. There is evidence that other genes await to be discovered on chromosomes 5 and 6 [54,55]. Some of those genes may actually predispose to autoimmunity in general. This would explain the increased prevalence of autoimmune diseases like type I diabetes in CD patients [56]. Given the role of Th1 immunity in autoimmune disease some of these genes may shift the Th1/Th2 balance, influence the regulatory T cell circuit or be in other ways involved in the maintenance of (oral) tolerance. Given the fact that women have a twofold increased risk of CD compared to men, the hormonal status is also likely to play a role [57]. It will be a challenge for the coming years to unravel this intricate interplay between environmental and genetic factors.

**Potential for prevention and therapy**

The rapid progress in our understanding of the mechanisms underlying CD potentially allows the development of novel strategies for disease prevention and alternative therapies. With the identification of the gene variants that predispose to CD it may become possible to identify individuals at high risk, particularly in

**Table 1.** The most important factors contributing to the development of celiac disease.

| Factors contributing to the onset of CD | Mechanism   |
|---|---|
| Gluten                                  | <ul style="list-style-type: none"> <li>• Elicits T cell responses,</li> <li>• Induces cytokine production and intestinal lesions</li> </ul>                             |
| HLA-DQ2 or HLA-DQ8                      | <ul style="list-style-type: none"> <li>• Gluten presentation</li> </ul>   |
| MYO9B                                   | <ul style="list-style-type: none"> <li>• Increased permeability of the intestine?</li> </ul>  |
| Pro-autoimmune genetic background       | <ul style="list-style-type: none"> <li>• Shift in Th1/Th2 balance towards Th1</li> <li>• Defect in generation of active tolerance (e.g. regulatory T cells),</li> </ul> |
| Viral infections                        | <ul style="list-style-type: none"> <li>• IFN production,</li> <li>• Tissue damage</li> </ul>  |
| Tissue damage                           | <ul style="list-style-type: none"> <li>• Increased level of tTG,</li> <li>• Danger signals</li> </ul>   |
| Early termination of breastfeeding      | <ul style="list-style-type: none"> <li>• Decreased protection against infections</li> </ul>   |
| Gender                                  | <ul style="list-style-type: none"> <li>• Hormone-related pro-autoimmune status</li> </ul>   |

families in which one member is already affected. Obviously, disease could be prevented in such individuals by not introducing gluten into the diet but more subtle approaches may also have a big impact. As mentioned earlier, the introduction of large gluten amounts into the infants' diet significantly increased the incidence of CD in Sweden. Conversely, a more gradual introduction of lower amounts of gluten into the diet may help the immune system to cope with the dietary proteins that are clearly strong immunogens. There is also evidence that introduction of gluten while breastfeeding has beneficial effects, which may at least partially result from reinforced protection to pathogenic microorganisms due to maternal IgA antibodies in the breast milk. Such approaches could thus effectively prevent CD and should be investigated for their efficacy.

Moreover, several alternatives to a lifelong gluten-free diet are now being studied. The use of a bacterial prolyl oligopeptidases for degradation of gluten into harmless fragments has been proposed [58]. Unfortunately, these bacterial enzymes are susceptible to pepsin and lack activity at low pH, which makes them unsuitable as an oral supplement for degradation of gluten in the stomach. Recently, however a novel prolyl endoprotease from yeast has been described that does not suffer from these limitations [59] and could degrade gluten in the stomach and prevent the activation of gluten specific T cells in the duodenum. Another possibility of reducing the immunostimulatory properties of gluten could be achieved by inhibiting tTG. Such inhibitors would have to be more specific and powerful than the ones available [60] but their application may be limited as tTG is known to participate in tissue damage repair and the issue of safety should thus be addressed.

Interfering with the binding of gluten peptides to HLA-DQ molecules is another option. Specific HLA-DQ blockers would selectively target HLA-DQ2 and DQ8 molecules and leave other HLA-molecules intact. Such an approach may therefore be safe but it will be a challenge to design an effective blocker. In addition, various other approaches have been proposed such as blocking the proinflammatory cytokine IL-15 [27,48] and treatment with IL-10 [61]. It is doubtful, however, if a patient would be prepared to undergo such treatments, with many potential side effects, when a perfectly safe gluten-free diet is an effective alternative. Finally, the generation of safer foods would be of great benefit to patients. Work is in progress to identify wheat varieties with relatively low immunogenicity [44,62,63]. Such wheat varieties could form the basis of a dedicated breeding program to obtain wheat that lacks the immunogenic gluten peptides. Another approach would rely on the generation of artificial gluten genes from which T cell stimulatory epitopes have been removed and the introduction of such genes in non-toxic cereals like rice or maize. Due to diversity of wheat gluten and the abundance of immunogenic gluten sequences, however, neither of these approaches will be easy. On the short term the

identification of alternative cereals that are safe for CD patients, like the Ethiopian cereal Teff [64], is likely to provide valuable additions to the diet of patients.

## **CONCLUDING REMARKS**

In recent years we have obtained detailed insight into the interaction between HLA-DQ, gluten and T cells in CD. This is the first example of an HLA-associated disorder where the role of HLA in the disease has been firmly established. Since CD shares features with other HLA-associated autoimmune disorders, such as type I diabetes and rheumatoid arthritis, this knowledge may be useful to unravel the pathological mechanisms in those diseases as well. In this respect it is noteworthy that the posttranslational modification of gluten by tTG has a counterpart in rheumatoid arthritis where deimination of arginine to citrulline (citrullination) in proteins like fillagrin, fibrin or vimentin evokes a highly specific and predictive antibody formation to these citrullinated proteins [65]. Likewise, citrulline residues were also detected in myelin basic protein (MBP), a common autoantigen in multiple sclerosis and such modified MBP elicited stronger CD4<sup>+</sup> T cell responses compared to the unmodified one [66]. Posttranslational modification may thus be a common denominator in autoimmune diseases.

But the grand challenge is still awaiting CD-researchers: to understand why disease develops in only a minority of HLA-DQ2 positive individuals. Are unrelated events, like enteroviral infections, responsible for loss of tolerance or do patients have a genetic make-up that will lead to disease development anyway? In our opinion many combinations of these two options are possible. The child that develops CD directly after the first introduction of gluten in the diet is likely to have a different genetic make-up as the individual that has eaten gluten without problems for 50 years but now develops CD. Are viral infections the incriminated environmental factors directly triggering the onset? To address this question it would be ideal, if we had an animal model. Unfortunately, such a model still does not exist. For the time being we will therefore continue to work with patients. Fortunately, there is a return of investment for them: with the knowledge we have we can work towards novel solutions that will make it easier to live with and handle celiac disease.

## **SCOPE OF THIS THESIS**

Celiac disease is a common disorder of the small intestine caused by intolerance to gluten, proteins found in wheat and related cereals. In this study two major aims

were explored: 1) which specific properties of gluten contribute to its disease-inducing characteristics and 2) how can gluten toxicity be avoided.

Approximately 95% of celiac patients express HLA-DQ2. This has been elucidated by showing that HLA-DQ2 molecules can bind and present gluten-derived peptides to gluten-specific T cells in the small intestine, which results in inflammation and the clinical symptoms associated with celiac disease. In order to provide an explanation for the unique gluten binding properties of HLA-DQ2 we eluted, sequenced and analyzed a large number of autologous peptides displayed in HLA-DQ2 and compared them with the set of T cell stimulatory gluten-derived epitopes. The results indicate that HLA-DQ2 has several characteristics that explain why it can specifically interact with a large array of distinct gluten peptides (Chapter 2).

We also observed that some of the gluten peptides are being recognized by gluten-specific T cells in quite an unusual way, requiring the presence of a proline at position p-1. We investigated the molecular basis for this phenomenon and addressed the issue of its potential importance in the pathomechanism of celiac disease (Chapter 3).

It is well established that not only wheat gluten must be excluded from the celiac diet, but also the consumption of rye, barley and sometimes oats can also result in occurrence of similar symptoms. To investigate which peptides from those cereals might be responsible we identified several sequences in gluten-like proteins of barley, rye and oats that were able to stimulate gluten-specific T cells from celiac disease patients. We concluded that the disease-inducing properties of these cereals could be explained by cross-reaction of the gluten-specific T cells with the homologous peptides in other grains (Chapter 4).

Celiac disease is a complex multifactorial disease in which both environmental and genetic factors contribute to the onset. It is estimated that HLA phenotype constitutes only about 40-50% of the genetic background while many other genes are most likely implicated. Genome-wide linkage studies indicated the gene coding for human prolyl oligopeptidase as a potential candidate. Prolyl oligopeptidases are enzymes that have been shown to be capable of degrading gluten molecules. A defect in the gene product might thus potentially contribute to celiac disease development. However, investigation at both the genetic and protein level provided no evidence for a role of this gene in disease development (Chapter 5).

At present the only available treatment for celiac patients is a strict adherence to a gluten-exclusion diet. This is not only expensive and burdensome but also very difficult as the normally gluten-free foods are often contaminated with gluten. Development of non-toxic grains without compromising the unique baking properties of gluten and similar proteins would improve the quality of life of millions of celiac patients worldwide. In this thesis we propose a rational strategy, which by means of single-nucleotide targeted mutagenesis, raises the possibility of gluten detoxifica-

tion (Chapter 4). Another approach aiming at the improvement of the quality of life of celiac patients is based on the concept of *in vivo* destruction of toxic gluten sequences by an orally administered enzyme. We extensively investigated the potential of prolyl endoprotease from *Aspergillus niger* for this purpose and were able to demonstrate that this enzyme is a prime candidate for a clinical trial aimed at *in vivo* degradation of gluten (Chapter 6).

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