REVIEW

Celiac disease: how complicated can it get?

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Abstract In the small intestine of celiac disease patients, dietary wheat gluten and similar proteins in barley and rye trigger an inflammatory response. While strict adherence to a gluten-free diet induces full recovery in most patients, a small percentage of patients fail to recover. In a subset of these refractory celiac disease patients, an (aberrant) oligoclonal intraepithelial lymphocyte population develops into overt lymphoma. Celiac disease is strongly associated with HLA-DQ2 and/or HLA-DQ8, as both genotypes predispose for disease development. This association can be explained by the fact that gluten peptides can be presented in HLA-DQ2 and HLA-DQ8 molecules on antigen presenting cells. Gluten-specific CD4$^+$ T cells in the lamina propria respond to these peptides, and this likely enhances cytotoxicity of intraepithelial lymphocytes against the intestinal epithelium. We propose a threshold model for the development of celiac disease, in which the efficiency of gluten presentation to CD4$^+$ T cells determines the likelihood of developing celiac disease and its complications. Key factors that influence the efficiency of gluten presentation include: (1) the level of gluten intake, (2) the enzyme tissue transglutaminase 2 which modifies gluten into high affinity binding peptides for HLA-DQ2 and HLA-DQ8, (3) the HLA-DQ type, as HLA-DQ2 binds a wider range of gluten peptides than HLA-DQ8, (4) the gene dose of HLA-DQ2 and HLA-DQ8, and finally,(5) additional genetic polymorphisms that may influence T cell reactivity. This threshold model might also help to understand the development of refractory celiac disease and lymphoma.

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With a prevalence of 1% in western populations, celiac disease (CD) is one of the most common inflammatory disorders of the small intestine (Green and Cellier 2007). CD is often assumed to have its onset in childhood, but it has recently been suggested that adults can also develop CD (Vilppula et al. 2009). Clinical manifestations vary according to age group: infants and young children present with diarrhea, abdominal distention, and failure to thrive, whereas adults that develop CD not only present with diarrhea, but also with silent manifestations such as anemia, osteoporosis, or neurological symptoms (Green and Cellier 2007). Immunohistochemistry of the small intestine of patients shows villous atrophy, crypt hyperplasia, and elevated levels of intraepithelial lymphocytes (IELs). The only therapy until now is a gluten-free diet, which will normalize the clinical and histological manifestations and allows the patients to live an otherwise normal life.

A small percentage of adult-onset CD patients develop a primary or secondary resistance to a gluten-free diet (Fig. 1). This condition is called refractory celiac disease (RCD) and is characterized by persisting villous atrophy and elevated levels of IELs. Currently, RCD is subdivided into two subtypes: RCD type I (RCD I) and RCD type II (RCD II) that both display clinical and histological resistance to a gluten-free diet (Daum et al. 2005). RCD II, however, is associated with the presence of an aberrant IEL population that lacks surface T cell receptor (TCR)-CD3 expression, but contains intracellular CD3ε and has clonal TCRγ-gene rearrangements (Cellier et al. 1998). These aberrant IELs can gain chromosomal abnormalities and develop into surface TCR-CD3$^-$ lymphoma cells (Deleeuw et al. 2007; Verkarre et al. 2003). RCD II is
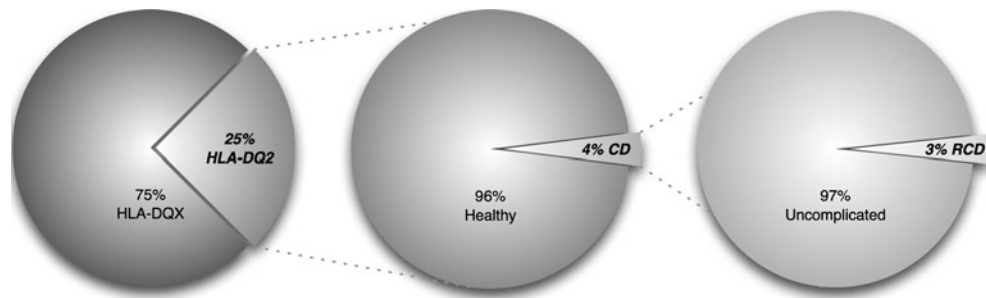
Fig. 1 Prevalence of CD and complicated CD in the Caucasian population. Approximately 25% of the general Caucasian population is HLA-DQ2$^+$. From these genetically susceptible individuals, only 4% develop CD. In majority of the CD patients, the disease course is therefore considered a premalignant condition, and roughly 50% of the RCD II patients develop overt lymphoma within 5 years of diagnosis (Al-Toma et al. 2007; Malamut et al. 2009). In summary, the majority of CD patients has an uncomplicated disease course and can be treated with a gluten-free diet (Fig. 1). RCD II and RCD-associated lymphoma, however, are difficult to treat and have therefore poor 5-year survival rates of <44% and <20%, respectively (Al-Toma et al. 2007).

uncomplicated. Roughly 3% of the CD patients will not respond to a gluten-free diet and develop RCD. A subset of RCD patients develop RCD II of which approximately 50% develop RCD-associated lymphoma (not shown)

The development of CD is determined by both environmental and genetic factors. In the 1950s, ingestion of wheat products was described to cause malabsorption symptoms in patients (Dicke et al. 1953). Later on, it was established that gluten, the storage proteins in wheat, barley and rye, caused a cell-mediated immune response in the small intestine (Ferguson et al. 1975). In addition to this environmental factor, CD development involves genetic predisposition, as the vast majority of the CD patients possess human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8 (Spurkland et al. 1997). HLA-DQ2 is a genotype that is present in roughly 25% of the European population (Bourgey et al. 2007; Fig. 1). Yet, only ~4% of all HLA-DQ2$^+$ individuals develop CD (Fig. 1) (Wolters and Wijmenga 2008). CD development in HLA-DQ2- and HLA-DQ8-negative individuals is extremely rare (Karell et al. 2003). These findings came together with the observation that CD4$^+$ T cell lines from duodenal biopsies of CD patients specifically respond to gluten peptides presented by HLA-DQ2 and/or HLA-DQ8 (Lundin et al. 1993, 1994). The etiology of RCD is much less clear, but seems to be associated with HLA-DQ2 (Al-Toma et al. 2006).

In short, the complex interplay of multiple genetic and environmental factors will determine the development of CD and its complications. This review describes current knowledge on gluten, HLA-DQ, and the immunopathogenesis of CD and its complications. In addition, we present a likely sequence of events in the development of CD and discuss the factors that may influence the risk of CD development.

Gluten: the disease-inducing environmental trigger

The visco-elastic properties of gluten are essential for dough formation of wheat flour and give bread its unique texture and taste. Because of its unique properties, gluten is widely used in the food industry: not only in products that are readily associated with wheat, like bread, cookies and pasta, but also as a hidden ingredient in sauces, instant soups, and even medication. Consequently, the daily gluten intake on a gluten-containing diet in Western Europe and the United States is high, between 15 and 20 g per day. The omnipresence of gluten makes adherence to a gluten-free diet challenging for CD patients.

Gluten is a heterogeneous mixture of gliadins and glutenins in wheat or similar proteins in barley and rye. Each wheat variety expresses multiple α-, γ-, and ω-gliadins in addition to low- and high-molecular weight glutenins. Gluten has a very high content of the amino acids glutamine (30%) and proline (15%). By virtue of its high glutamine content, gluten is rich in nitrogen, an essential factor for seed germination. The high proline content renders gluten highly resistant to degradation by gastrointestinal enzymes, making it possible for large immunogenic gluten peptides to reach the mucosal surface (Shan et al. 2002, 2005). As modern wheat varieties contain three complete genomes encoding gliadins and glutenins, up to 100 different gluten proteins may be present in a single wheat variety, and many of these are implicated in the pathogenesis of CD.

HLA-DQ: the strongest disease-associated gene locus by far

The strong genetic influence in CD is apparent, as the concordance between monozygotic twins is 80%, whereas in dizygotic twins, this is only 11% (Nistico et al. 2006), which is approximately the same as the risk for first-degree relatives (Dube et al. 2005). The main genetic influence in CD is

HLA, which was first indicated by studies describing the predominance of HLA-B8 and HLA-DR3 serotypes in CD patients (Falchuk et al. 1972; Keuning et al. 1976). Later studies established that the strongest association is with HLA-DQ2 (DQA*0501, DQB*0201, termed HLA-DQ2.5 hereafter) (Sollid et al. 1989), which is encoded together with HLA-B8 and HLA-DR3 on the highly conserved ancestral haplotype 8.1 (Price et al. 1999). CD is associated, to a lesser extent, with HLA-DQ8 (DQA*03, DQB*0302) (Spurkland et al. 1997). The strong association between HLA-DQ2.5 and CD is further illustrated by the observation that individuals homozygous for HLA-DQ2.5 have a fivefold increased risk for development of CD compared to individuals heterozygous for HLA-DQ2.5 (Mearin et al. 1983). Similarly, HLA-DQ 2.5 homozygosity is associated with the development of RCD II and RCD-associated lymphoma, whereas this association is less clear for HLA-DQ2.5 heterozygosity and HLA-DQ8 (Al-Toma et al. 2006). Another HLA-DQ2 variant exists: HLA-DQ2.2 (DQA*0201, DQB*0202), which has a peptide-binding motif that is almost identical to that of HLA-DQ2.5 (van de Wal et al. 1997). Whereas HLA-DQ2.5 predisposes to CD, HLA-DQ2.2 does not. This difference is related to the peptide-binding properties of these HLA-DQ2 variants (see below). The estimated risk effect of HLA-DQ2 and HLA-DQ8 on CD development is estimated to be ~35% (Hunt et al. 2008).

Not all HLA-DQ2.5$^+$ and HLA-DQ8$^+$ individuals develop CD, indicating that these HLA genotypes are necessary, but not sufficient for CD development. Recent large-scale genetic association studies identified many additional genetic loci that all make a small contribution to the risk to develop CD (Box 1 and Table 1). Importantly, most of these genes encode proteins involved in immunity, which supports the notion that CD is an immune-related disorder and possibly provides clues on the immunopathogenesis of CD.

The adaptive immune response: gluten, HLA-DQ, and CD4$^+$ T cells

As noted above, CD develops almost exclusively in HLA-DQ2.5$^+$ or HLA-DQ8$^+$ individuals. It is also well established that in CD patients, gluten-derived peptides presented by either HLA-DQ2.5 or HLA-DQ8 induce a CD4$^+$ T cell response. Both HLA-DQ2.5 and HLA-DQ8 prefer to bind peptides with negatively charged amino acids at anchor residues. Gluten peptides, however, are virtually devoid of negative charges, and native gluten peptides thus bind poorly to HLA-DQ2.5 or HLA-DQ8. Consequently, CD4$^+$ T cells specific for native gluten peptides are rare. It has become clear that the enzyme tissue transglutaminase 2 (TG2) can modify gluten peptides to fit the requirements for high affinity binding to HLA-DQ2 and HLA-DQ8 (Molberg et al. 1998; van de Wal et al. 1998a). TG2 can convert non-charged glutamine into negatively charged glutamic acid, a process called deamidation. Because gluten has a high content of glutamine (Q) and proline (P), the sequences QP, QXP and QXXP (in which X can represent any amino acid) are frequently found in gluten peptides. Strikingly, only in the sequence QXP that the glutamine is converted, which results in highly selective introduction of negative charges in gluten peptides (Vader et al. 2002). This specific deamidation process introduces the negative charges at the positions favored by HLA-DQ2 and HLA-DQ8, thereby expanding the presentable gluten peptide repertoire (Fig. 2). As a result, the gluten-specific CD4$^+$ T cell repertoire is substantially expanded, which enhances the inflammation and disease development (Fig. 2).

TG2 is mostly retained intracellularly in an inactive form and is activated upon its release during tissue damage (Lorand and Graham 2003; Siegel et al. 2008). Therefore, something should trigger tissue damage which initiates TG2 release, allowing the modification of gluten peptides. Whereas CD4$^+$ T cell responses against native gluten

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Box 1 Non-HLA genes associated with CD

Candidate gene association study: Candidate genes, selected on the basis of current understanding of CD immunopathology, were tested for association with CD. Genes studied with this approach include, among others, IFN-γ, FAS, TCR, and TG2 (van Heel et al. 2005). No convincing association with CD was found.

Genetic linkage study: This approach is aimed at the identification of chromosomal regions that likely contain disease-causing genes in families with a high prevalence of CD. The genomic region 2q33 showed linkage to celiac disease in multiple populations. This region contains the genes CD28, CTLA4, and ICOS which all control different aspects of the T cell response (van Heel et al. 2005). Linkage was also found for chromosome 5q31-33 (Greco et al. 2001) and chromosome 19p13.1 (van Belzen et al. 2003). Linkage to these regions, however, could not always be replicated in other populations.

Genome-wide association study: In recent years, it has become possible to perform large-scale case control-based association studies using single nucleotide polymorphisms. With this approach, it is possible to identify common variants in the genome that predispose to disease. Until now, ten non-HLA loci associated with CD have been identified and linkage to 2q33 has been confirmed (Table 1) (Hunt et al. 2008;Trynka et al. 2009;van Heel et al. 2007). Recently, 13 additional true risk variants and 13 suggestive risk variants were identified (Dubois et al. 2010). Although causality has only been proven for the risk allele SH2B3 (Zhernakova et al. 2010), it is clear that nearly all associated regions contain genes involved in immune response.

Table 1 Loci associated with CD development

| Locus	Gene candidate	Function	Odds ratio[a]
6p21	HLA	Antigen presentation	6.23 (5.95–6.52)
3q25-3q26	IL12A	Subunit of IL12, regulates Th1 differentiation	1.36 (1.29–1.44)
3p21	CCR1, CCR2, CCR3 and CCR5	Recruitment of immune cells to the site of inflammation	1.30 (1.23–1.39)
3q28	LPP	Possible role in maintaining cell shape	1.29 (1.25–1.34)
6q23	TNFAIP3	Inhibits NFκB activation and TNF-mediated apoptosis	1.23 (1.17–1.28)
12q24	SH2B3	Adaptor molecule involved in signaling in T cells	1.20 (1.15–1.24)
2q11-2q12	IL18R1 and IL18RAP	Respectively the α and β-chain of IL18 receptor, IL18 is a pro-inflammatory cytokine	1.19 (1.14–1.25)
6q25	TAGAP	Role in modulating cytoskeletal changes	1.16 (1.12–1.21)
2p16	REL	Component of NFκB transcription complex	1.15 (1.11–1.20)
2q33	CTLA4	Inhibitory effect on the T cell response	1.14 (109–1019)
	CD28	Stimulating effect on the T cell response	
	ICOS	Stimulating effect on the T cell response	
1q31	RGS1	Acts as GTPase activating protein, thereby regulating cell signating	0.80 (0.76–0.84)
4q27	IL2	Stimulating proliferation of T cells	0.74 (0.70–0.78)
	IL21	Regulates the function of T and NK cells	

[a] Odds ratios from (Dubois et al. 2010)

peptides are relatively rare, they could represent the first breach in oral tolerance to gluten. The presentation of native gluten peptides by HLA-DQ2 or HLA-DQ8 to CD4^{+} T cells will lead to the production of IFN-γ (Fig. 2). IFN-γ will in turn lead to higher expression of the HLA-DQ molecules and thereby, to increased gluten peptide presentation (Fig. 2). In the presence of gluten, this could become a self-amplifying loop that could cause limited tissue damage locally. This tissue damage would lead to the release of TG2 that will modify native gluten peptides into high affinity ligands for HLA-DQ2 and/or HLA-DQ8, thereby expanding the gluten-specific CD4^{+} T cell responses and leading to additional tissue damage: the initiation of a second self-amplifying loop (Fig. 2). Alternatively, infections occurring in the gastrointestinal tract would generate a pro-inflammatory milieu that might lead to loss of tolerance to native gluten peptides and generate tissue damage simultaneously and thus, initiate deamidation by TG2.

Activated intraepithelial lymphocytes damage the intestinal epithelium

Intraepithelial lymphocytes are localized between intestinal epithelial cells at the basolateral side of the epithelium and are thought to play an important role in immunosurveillance of the epithelium. The IEL population in the small intestine is a mixed population of TCRαβ^{+} T cells, TCRγδ^{+} T cells, and NK cells, although the vast majority
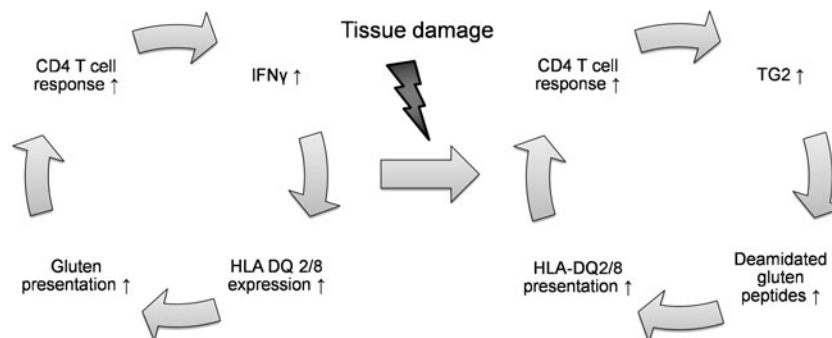

Fig. 2 Self-amplifying loops in the development of CD. HLA-DQ2^{+} or HLADQ8^{+} individuals can present native gluten peptides on their APC to CD4^{+} T cells. The IFN-γ released in this process can in turn boost HLA-DQ2/8 expression. This low-grade inflammation might eventually lead to tissue damage with TG2 release. TG2 is able to expand the presentable gluten peptide repertoire by deamidation. As a result, the CD4^{+} T cell response is strongly enhanced, leading to more IFN-γ, tissue damage, and increased release of TG2. The end result is full-blown CD

of the IELs are CD8^{+} TCRαβ^{+} T cells (Jabri and Ebert 2007). Furthermore, most of these TCR^{+} IELs express a variety of NK cell receptors that is distinct from the NK cell receptors expressed on blood T cells (Jabri et al. 2000). The NK cell receptors are thought to act mainly as T cell co-stimulators, lowering the threshold for T cell activation in stressful times (Bauer et al. 1999).

In active CD, the number of CD8^{+} TCRαβ^{+} and TCRγδ^{+} IELs is markedly increased. It is unclear whether this is a response to changes in the homeostasis of the epithelium, or a consequence of the pro-inflammatory milieu created by the CD4^{+} T cell response in the lamina propria (Fig. 3). Similar to IELs of normal controls, IELs from active CD express NK cell receptors. When compared to normal controls, however, IELs from CD patients have acquired a more activating NK cell receptor repertoire (Jabri et al. 2000). In the healthy small intestine, IELs predominantly express the inhibitory CD94/NKG2A receptor. In contrast, IELs in CD express high levels of activating receptors like CD94/NKG2C and NKG2D (Meresse et al. 2004, 2006). Simultaneously, intestinal epithelial cells in CD upregulate MIC and HLA-E, the ligands for NKG2D and CD94/NKG2C, respectively. Interaction of NKG2D and CD94/NKG2C with their ligands will enhance IFN-γ production and cytolysis, leading to tissue damage (Fig. 3). An important factor in acquiring an activating NK cell receptor repertoire is interleukin 15 (IL-15; Box 2), which has been shown to upregulate both NKG2D and CD94/NKG2C on IELs of active CD patients and boost their ability to lyse enterocytes (Meresse et al. 2004, 2006). In addition, IL-15 can alter the NK cell receptor function, leading to NK cell receptor-mediated cytotoxicity indepen-
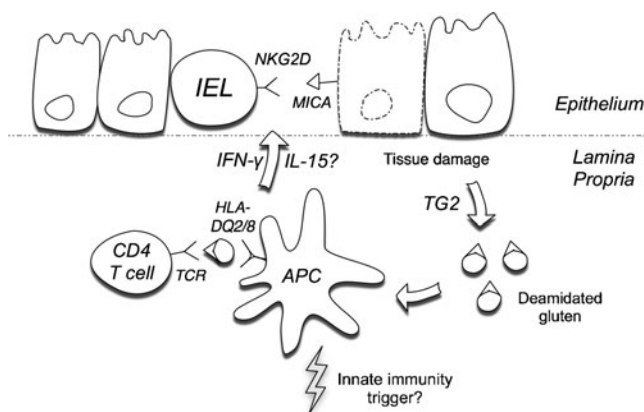
Fig. 3 Crosstalk between the CD4^{+} Tcell response and IEL cytotoxicity. The CD4^{+} T cell response to gluten may lead to IFN-γ production and potentially to upregulation of IL-15, which in turn boosts IEL-mediated cytotoxicity. Activated IELs lyse the epithelium, which leads to TG2 release and subsequent deamidation of gluten peptides. This may constitute yet another self-amplifying feedback loop, as deamidation of gluten peptides will enhance the CD4^{+} T cell response (see Fig. 2)

Box 2 IL-15

IL-15 is a cytokine that, just as IL-2, is able to induce T cell proliferation, IFN-γ production, and cytotoxicity. Furthermore, IL-15 is known to play an important role in NK cell development and activation (Fehniger and Caligiuri 2001). Under normal circumstances, IL-15 expression is strictly regulated at the level of transcription, translation, and secretion (Budagian et al. 2006). In CD, this regulation is disrupted, which results in massive upregulation of IL-15 in the epithelium and lamina propria. The abnormal availability of IL-15 results in chronic inflammation by survival, proliferation, and activation of IELs (Di Sabatino et al. 2006;Ebert 1998). Furthermore, IL-15 can exert an inhibitory effect on TGF-β, a negative regulator of the immune response (Benahmed et al. 2007). Recently, it has been shown that IL-15 can synergize with IL-21, a cytokine expressed on CD4^{+} T cells and a stimulator of IFN-γ production and cytolytic activity of CD8^{+} T cells and NK cells (Ebert 2009;Parrish-Novak et al. 2000).

dent of TCR specificity (Meresse et al. 2004, 2006). In conclusion, while gluten-specific CD4^{+} T cells elicit an inflammatory response in the lamina propria, IELs in the epithelium acquire activating NK receptors and the ability to lyse stressed epithelial cells independent of T cell receptor signaling, which likely contributes to the typical tissue damage in CD.

In RCD, the survival, expansion, and acquisition of an NK cell-like phenotype by IELs is even more pronounced than in CD, possibly as a result of the presence of larger amounts of IL-15. RCD II patients have an aberrant clonal IEL population that lacks surface TCR-CD3 expression. Studies on aberrant TCR-CD3^{-} IEL lines from RCD II patients showed that, upon stimulation with IL-15, these cell lines express granzyme B and lyse the intestinal epithelial cell line HT29, suggesting a role for aberrant IELs in perpetuating epithelial damage in RCD II (Mention et al. 2003). Therefore, IL-15-dependent NK cell-like transformation of IELs may be an essential step in the immunopathology of RCD.

A threshold model for the risk of CD development

The expansion of the presentable gluten peptide repertoire due to the release and activity of TG2 is a critical step in the pathogenesis of full-blown CD. Several lines of evidence support the notion that the level of gluten presentation to T cells critically influences the risk of disease development.

First, HLA-DQ2.5 homozygous individuals have a fivefold higher risk of CD development than HLA-DQ2.5 heterozygous individuals (Mearin et al. 1983). This gene dose effect directly correlates with the magnitude of the CD4^{+} T cell response: antigen presenting cells (APC) from HLA-DQ2.5 homozygous individuals induce very strong proliferative T cell responses and IFN-γ production, while APC from HLA-DQ2.5/DQX heterozygous individuals

induce much weaker responses (Vader et al. 2003b). These data indicate that the number of HLA-DQ2.5 molecules capable of presenting gluten peptides on the surface of APC will define the magnitude of the CD4$^+$ T cell response.

Second, whereas HLA-DQ2.5 is associated with CD development, the homologous HLA-DQ2.2 is not. Although these two variants have almost identical peptide-binding motifs, HLA-DQ2.2 can only bind a subset of the gluten peptides that can bind to HLA-DQ2.5. This difference is explained by the fact that a proline at position 3 in peptides has an adverse effect on peptide binding to HLA-DQ2.2 (van de Wal et al. 1997). As gluten epitopes cluster in proline-rich regions (Arentz-Hansen et al. 2002), many gluten peptides have a proline at position 3 and do not bind to HLA-DQ2.2 (Vader et al. 2003b). Consequently, HLA-DQ2.5 is able to present a much broader repertoire of gluten peptides than HLA-DQ2.2. In addition, HLA-DQ2.5 is better at retaining gluten peptides in its binding groove compared to HLA-DQ2.2 (Fallang et al. 2009). As a result, gluten peptide presentation by HLA-DQ2.5 is protracted compared to presentation by HLA-DQ2.2, which will increase the chance for productive CD4$^+$ T cell stimulation.

Third, CD is associated mainly with HLA-DQ2.5 and, to a lesser extent, with HLA-DQ8. Although a variety of gluten peptides has been identified that can stimulate HLA-DQ8 restricted T cells from CD patients, one α-gliadin peptide in particular appears to be immunodominant, as this peptide invariably induces specific T cell responses in HLA-DQ8$^+$ CD patients (Henderson et al. 2007; Tollefsen et al. 2006; van de Wal et al. 1998b, 1999; Kooy et al. unpublished data). In contrast to the HLA-DQ2.5 restricted α-gliadin peptides, the HLA-DQ8 peptide is not derived from a proline-rich region of the α-gliadin protein, and therefore likely susceptible to degradation in the gastrointestinal tract. Furthermore, whereas for HLA-DQ2 a single deamidation in a gluten peptide is sufficient to evoke a CD4$^+$ T cell response, for HLA-DQ8, deamidation at two positions is preferred (Henderson et al. 2007), which may limit the generation of strong antigenic gluten peptides. The fact that the immunodominant HLA-DQ8 peptide is more readily degraded and requires more deamidation steps, limits the availability for antigen presentation and may therefore limit the risk to develop CD.

Fourth, further evidence that the level of gluten presentation is a critical parameter comes from a totally different angle: most CD patients tolerate oat even though it has been shown that the gluten-like molecules in oat can elicit CD4$^+$ T cell responses in CD patients (Arentz-Hansen et al. 2004; Vader et al. 2003a) There are two striking differences between the relatively safe oat and the disease-inducing cereals wheat, barley, and rye: (1) while the gluten-like molecules in oat contain only two antigenic sequences, dozens are found in gluten and the gluten-like

molecules of barley and rye, (2) the "gluten" content of oat is much lower compared to the other cereals. Consumption of oat thus results in a much lower exposure to antigenic peptides, in comparison with the other cereals, and this is apparently tolerated, as it does not lead to disease in the majority of patients.

Collectively, these data indicate the presence of a threshold to develop CD. Initiation of CD becomes more likely with increased T cell exposure to gluten antigens. This exposure is influenced by the type and amount of HLA-DQ, as this determines the efficiency of gluten peptide presentation to CD4$^+$ T cells. For HLA-DQ2.5 homozygous individuals, the threshold to develop CD is most easily exceeded, whereas for HLA-DQ2.2$^+$ and HLA-DQ8$^+$ individuals, the threshold is much higher.

CD development: a series of unfortunate events

The past two decades have witnessed the identification of several critical immunological factors in CD, from which a likely sequence of events in the development of this disease can be deduced. It is well known that healthy individuals can have antibodies against native gluten peptides. As an antibody response is controlled by CD4$^+$ T cell help, such individuals most likely have CD4$^+$ T cells specific for native gluten peptides, indicating that the mere presence of such T cells is, in general, not sufficient to exceed the threshold to develop CD (Fig. 4). This indicates that in the majority of individuals, tolerogenic and regulatory processes in the intestine keep gluten-specific T cell responses in check. This steady state can be breached by frequent episodes of enteroviral infections, as this leads to the secretion of inflammatory cytokines and differentiation of Th1 cells (Stene et al. 2006), thereby enhancing the response to gluten (Fig. 4). Subsequently, the combined effect of low-level gluten reactivity and pathogen-induced inflammation could lead to tissue damage and the release of TG2. In turn, the activity of TG2 would generate a large repertoire of deamidated gluten peptides with high affinity for HLA-DQ, thereby boosting the gluten-specific T cell response (Fig. 4). At this point in CD development, self-amplifying loops are in action: CD4$^+$ T cell responses against native gluten peptides lead to IFN-γ production to upregulation of HLA, and further amplification of the gluten-specific T cell response. TG2 released upon tissue damage expands the presentable gluten peptide repertoire, which will ultimately lead to more tissue damage (Fig. 2). Due to the massive expansion of the gluten-specific T cell pool, regulatory processes are no longer able to contain the T cell responses, and exposure to gluten suffices to perpetuate inflammation. Eliminating gluten from the diet is the only

Fig. 4 Threshold model for CD development. As HLA-DQ2.5 homozygous individuals can present more gluten peptides on their APC than HLA-DQ2.5 heterozygous individuals, HLA-DQ2.5 homozygotes have a higher risk of CD development a priori. In steady state conditions, presentation of native gluten peptides is unlikely to induce disease. This steady state can be breached by frequent viral infections and low-grade T cell responses to native gluten peptides that lead to release of TG2 upon tissue damage. TG2 activity will expand the presentable gluten peptide repertoire extensively, thereby increasing the risk to develop CD. The recently uncovered non-HLA genes associated with CD likely lower the threshold to develop CD
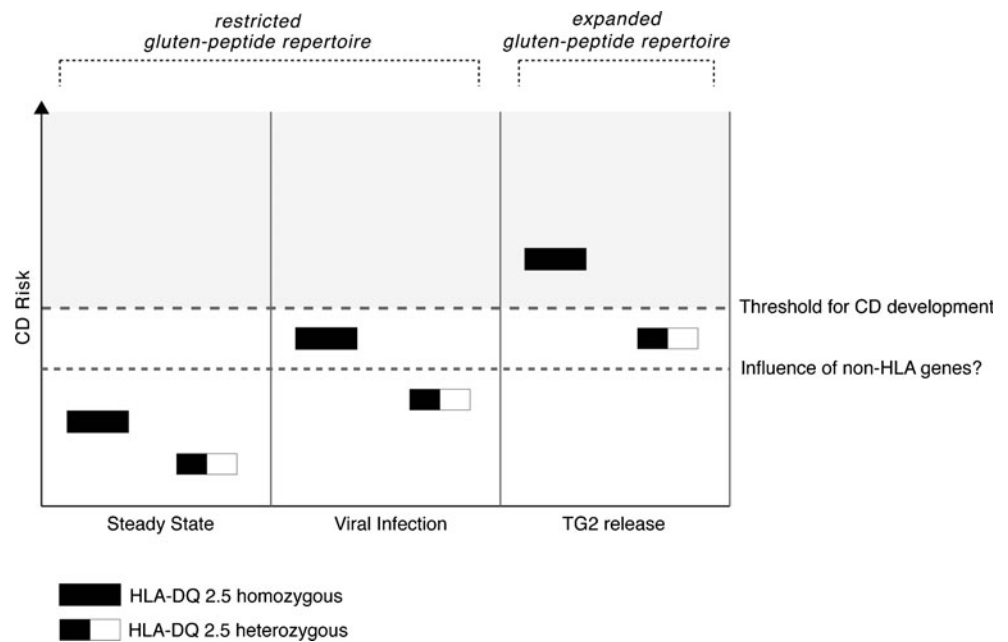
way to stop this process. At all stages in this scenario, the risk to develop CD is influenced by the HLA-DQ genotype, as HLA-DQ2.5 homozygous individuals will present more HLA-gluten complexes than HLA-DQ2.5 heterozygous individuals (Fig. 4). Simultaneously, an infiltrate of IELs is formed in the epithelium, which is potentially driven by the inflammatory milieu created by $CD4^+$ T cells in the lamina propria. These IELs upregulate activating NK cell receptors and acquire the ability to lyse enterocytes independent of the TCR.

The development of full-blown CD is most likely the result of an unfortunate series of events which, in isolation, would not lead to disease, but, combined, have a detrimental outcome. It is important to note that exposure to gluten, frequent enteroviral infections, and occasional TG2 activation likely occur in every individual, but usually do not result in CD development, even in $HLA-DQ2^+$ and/or $HLA-DQ8^+$ individuals (Fig. 1). It has now become clear

that the presence of a higher number of additional non-HLA risk alleles (Table 1) is directly correlated with an increase in the risk to develop CD (Romanos et al. 2009). This suggests that the influence of non-HLA genes lowers the threshold to develop CD and could skew the balance towards disease development (Fig. 4). It is plausible that non-HLA genes also increase the risk to develop complicated CD, although this hypothesis will be difficult to test as this patient population is very small. Thus, even though key pieces of the celiac puzzle have been collected and assembled, the picture is not yet complete.

Open questions

Although the molecular basis for the involvement of HLA-DQ in CD is now well established, a number of issues remain unclear.

Box 3 Direct effects of gluten

The role of gluten in the adaptive immune response in CD is well established. In addition, a direct (innate) effect of gluten on the intestinal mucosa has been suggested. One of the first indications for the potential of gluten to elicit a response in the epithelium came from in vivo challenges where administration of gliadin caused villous atrophy and increase of IELs within 2–3h after gluten ingestion (Ciclitira et al. 1984). This effect was later attributed to the non-immunodominant peptide p31-49 from alpha-gliadin (Sturgess et al. 1994). In vitro studies with p31-49 showed that epithelial alterations were independent of $CD4^+$ T cell activation. Furthermore, p31-49 stimulated IL-15 production in the lamina propria of cultured biopsies from CD patients (Maiuri et al. 2003). The fact that p31-49 could activate the local immune system implied that a receptor for p31-49 should exist. A transcellular transport pathway was proposed where anti-gliadin IgA antibodies were able to bind p31-49. This complex would then bind the transferrin receptor CD71 which would provide protected trafficking across the intestinal epithelium (Matysiak-Budnik et al. 2008). However, this mechanism would not function in all CD patients, as a relatively large fraction of them is IgA-deficient (McGowan et al. 2008).

We also assessed the hypothesis that a receptor for p31-49 is present on intestinal epithelial cells. Binding of p31-49 to intestinal epithelial cell lines, however, could not be detected, neither directly nor by either UV-crosslinking or TG2-induced transamidation (Tjon et al. unpublished data). In the absence of a receptor through which p31-43 could exert its activity, the molecular mechanism underlying the biological effects observed with this peptide remains unclear.

First, it is still controversial how immunogenic gluten peptides from the intestinal lumen reach the lamina propria where they can prime gluten-specific T cells. It has been suggested that gluten peptides can be transported during transient increased intestinal permeability during enteroviral infections (Stene et al. 2006) or by IgA-mediated retro-transcytosis (Matysiak-Budnik et al. 2008). Yet, this issue is far from resolved.

Second, a direct effect of gluten on the intestinal mucosa has been attributed to a peptide from α-gliadin, p31-49 (Box 3). It remains unclear, however, whether and how this peptide would exert its activity and contribute to the disease development (Box 3).

Third, TG2 is a crucial factor in expanding the presentable gluten peptide repertoire. In steady state conditions, TG2 is present in an inactive form intracellular and on the cell surface. An intriguing question is, therefore, how TG2 is activated and released in CD. We propose that TG2 is released upon tissue damage induced by the initial $CD4^+$ T cell response to native gluten peptides (Figs. 2 and 3). Alternatively, a recent study proposed a role for TLR3 ligands released during enteroviral infections that, upon ligation with TLR3, could result in TG2 activation (Siegel et al. 2008). These two possibilities are not mutually exclusive.

Fourth, in active CD, disrupted IL-15 regulation results in massive overexpression of IL-15. It remains unclear what causes this disruption. As on a gluten-free diet, the adaptive $CD4^+$ T cell response and IL-15 expression both decrease (Mention et al. 2003); it is possible that the adaptive $CD4^+$ T cell response has a direct effect on IL-15 expression (Fig. 3). Alternatively, innate signals delivered through TLRs may be responsible for elevated IL-15 levels.

Finally, the events leading from uncomplicated CD to RCD II and subsequent lymphoma development are still poorly understood. Lymphoma cells develop from the aberrant IELs in RCD II. One view is that aberrant IELs derive from mature TCR^+ IELs that have undergone oligoclonal expansion and lost surface TCR-CD3 expression due to overstimulation (Cellier et al. 1998). Alternatively, aberrant IELs could derive from a distinct population of $CD3^-$ $CD7^+$ precursor cells that can develop into T cells and NK cells (Gunther et al. 2005). In favor of the first hypothesis: although aberrant IELs lack surface TCR-CD3 expression, they do express CD3 intracellularly and display TCR-γ-gene rearrangements (Malamut et al. 2009). Furthermore, microarray analysis in one study on TCR-$CD3^+$ IEL lines from CD patients revealed a significant decrease in the transcript levels of TCRα- and TCRβ-chains (Meresse et al. 2006), indicating that IELs may lower TCR expression in CD. We found that aberrant IELs not only express $CD3\varepsilon$ intracellularly, but also have intracellular expression of the $CD3\gamma$, $CD3\delta$, and ζ-chains

(Tjon et al. 2008). In favor of the second hypothesis: the TCR chains were not always present (Tjon et al. 2008), and TCR rearrangements were often incomplete (Tjon et al. unpublished data). Furthermore, the full complement of CD3 chains and incomplete TCR rearrangements have also been observed in NK cell precursors, and even mature NK cells can carry partially rearranged TCRs. A recent study indicated that extrathymic TCR-gene rearrangement is an ongoing event in the human small intestine throughout life (Bas et al. 2009). This raises the possibility that aberrant IELs derive from cells in an early stage of extrathymic lymphocyte development.

Concluding remarks

Life used to be simple: CD was a rare disease, diagnosed in 1 in 1,000 individuals. Patients were HLA-DQ2$^+$ of HLA-DQ8$^+$ and could be treated effectively with a gluten-free diet. That was about it.

Now we know that CD affects ~1% of the population in Western Europe and the USA, most of which remain undiagnosed. Although good insight has been gained on the immunopathology of CD—inflammation in both lamina propria and epithelium—it remains unclear what triggers the development of CD and why not every patient is equally affected. In addition, with the recognition of RCD and RCD-associated lymphoma that do not respond to a gluten-free diet, CD has become a far more complicated disease.

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