Current Understanding of the Molecular and Cellular Basis of Celiac Disease

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Celiac disease has received increased attention in recent years. It has become evident that this is a common disorder in Western societies, affecting around 1:130 to 1:300 individuals (1). Celiac disease is an acquired disorder. It may be diagnosed in early childhood with classical symptoms such as diarrhea and malabsorption, but it may also be diagnosed later in life. In adults there is a broader spectrum of symptoms (1). Some have no subjective symptoms, some have the classical symptoms, but often the disease is manifested by anemia, osteoporosis, fatigue, depression, or infertility. The vague character of these symptoms does not suggest a gut disease, and persons who are diagnosed later in life often report protracted disease histories.

Celiac disease develops because of intolerance to ingested wheat gluten (consisting of the subcomponents gliadin and glutenin) or related proteins from rye and barley. There is chronic inflammation in the small intestine, with resultant flattening of the mucosa. The disease is effectively treated by a gluten-exclusion diet. Poor dietary compliance and undiagnosed disease are associated with long-term complications that include osteoporosis and an increased risk of intestinal lymphoma. Patients with celiac disease on a gluten-containing diet have increased levels of serum antibodies to various antigens including gluten and the autoantigen tissue transglutaminase (tTG) (2,3). The presence of antibodies to gluten and tTG is strictly dependent on dietary exposure to gluten.

Celiac disease belongs to the group of chronic inflammatory diseases of multifactorial etiology in which both genetic and environmental components are involved. Among these disorders, celiac disease is a particularly good model. In this chapter, I will outline briefly some recent advances in our understanding of this disorder.

GENETIC FACTORS IN CELIAC DISEASE

A high prevalence (10%) among first-degree relatives of patients with celiac disease indicates that susceptibility to the disease is strongly influenced by inherited (genetic) factors (4). Familial clustering is stronger in celiac disease than in most other chronic inflammatory diseases of multifactorial etiology (5,6). The strong genetic influence in celiac disease is further supported by a high concordance rate of 70% to
100% in monozygotic twins (7). Both HLA and non-HLA genes contribute to the genetic predisposition, and assuming a multiplicative model of disease genetics, it has been estimated that the overall importance of non-HLA genes is greater than that of HLA genes (5,6). These figures should be interpreted with caution, however, as increased sharing of environmental factors by the siblings would tend to overestimate the role of the non-HLA genes.

**HLA Genes**

Most patients with celiac disease carry the DRB1*0301-DQA1*0501-DQB1*0201 haplotype (the DR3, DQ2 haplotype) or are DRB1*11/12-DQA1*0505-DQB1*0301/DRB1*07-DQA1*0201-DQB1*0202 heterozygotes (carrying the DR5-DQ7/DR7-DQ2 haplotypes) (8–12). The DQα chains encoded by DQA1*0501 and DQA1*0505 differ by one residue in the leader peptide, and the DQβ chains encoded by DQB1*0201 and DQB1*0202 differ by one residue in the membrane proximal domain. These substitutions are highly unlikely to have any functional consequences. Patients with celiac disease with the above-mentioned DR–DQ combinations share the same functional DQ molecule on the cell surface, encoded by genes carried in the cis position (for example, DQA1*05 and DQB1*02 carried on the same haplotype) or the trans position (for example, DQA1*05 carried on a different haplotype to DQB1*02) (Fig. 1) (13).

Recombination (crossing over; rearrangement of genes on the same chromosome) is an important mechanism for the generation of HLA haplotypes (14). Accumulating evidence suggests that the DR3-DQ2, DR7-DQ2, and DR5-DQ7 haplotypes have a close evolutionary relationship. Fragments of DNA flanking the DQA1 gene of the

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**FIG. 1.** Patients with celiac disease who are DR3 or DR5/DR7 heterozygous express the same HLA-DQ2 molecule, HLA-DQ(α1*05, β1*02). The DQA1*05 and DQB1*02 genes are located in cis (on the same chromosome) in DR3 individuals, whereas they are located in trans (on opposite chromosomes) in DR5/DR7 heterozygous individuals. Figure adapted from Solllid and Thorsby (17), with permission.
DR3-DQ2 haplotype have been identified on the DR5-DQ7 haplotype, and fragments of DNA flanking the DQB1 gene of the DR3-DQ2 haplotype have been identified on the DR7-DQ2 haplotype (15,16). The genetic information in the DQ subregion of the DR3-DQ2 haplotype is thus reestablished in DR5-DQ7/DR7-DQ2 heterozygotes, although the sequence information is split between two chromosomes. Susceptibility to celiac disease therefore probably depends on an interaction between at least two genes on the DR3-DQ2 haplotype that are reunited in DR5-DQ7/DR7-DQ2 heterozygous individuals. The DQA1 and DQB1 genes are the primordial candidates because their products interact to form an HLA class II heterodimer and they are situated close to a putative recombination site.

Almost all the patients with celiac disease who are DQA1*05 and DQB1*02-negative bear the DRB1*04, DQA1*03, DQB1*0302 haplotype (that is, the DR4-DQ8 haplotype) and it is likely that these patients have an HLA association that is different from those who are DQ2-positive. Although it is less clear what is the primary disease susceptibility determinant of the DR4-DQ8 haplotype, most data favor DQ8 (17).

Overall, the existing data suggest that the susceptibility for developing celiac disease is primarily associated with two conventional DQ molecules DQ(α1*05,β1*02) (=DQ2) and to a lesser extent DQ(α1*03,β1*0302) (=DQ8). DQ molecules bind peptides and present these to CD4+ T helper cells carrying the αβ T-cell receptor (TCR). The genetic evidence thus points toward a central role CD4+ T cells in controlling the development of celiac disease.

CTLA4/CD28/ICOS Genes

Genome-wide linkage studies in celiac disease have indicated numerous susceptibility regions with weak genetic effects, and the indications are strongest for susceptibility genes located at 5qter and 11qter (18,19). However, no disease associations have been established for any genes of these regions. The only gene for which there are relatively consistent reports on disease association is the CTLA4 gene on chromosome 2q33. This has a single nucleotide polymorphism (A/G) at position 49 in exon 1, and the A allele has been found to be associated with celiac disease in both French and Swedish/Norwegian populations (20,21). In the Swedish/Norwegian material, there was also suggestive linkage for several markers, and in a Finnish study linkage was found for the marker D2S116, located immediately adjacent to CTLA4 (21). A study of Italian and Tunisian patients failed to confirm the association with the +49 polymorphism or linkage in the CTLA4 region (22). CTLA4 is involved in down-regulating T-cell responses, and the A allele of the +49 dimorphism is associated with increased CTLA-4 expression and enhanced control of T cell proliferation (23,24). Thus, the +49 dimorphism is a prime suspect for the observed genetic effect, although other polymorphisms in linkage disequilibrium cannot be ruled out. In this respect, it is worth noting that the CD28 and ICOS genes, whose gene products are central players in T- and B-cell activation, are located very close to the CTLA4 gene.
ENVIRONMENTAL FACTORS

Gluten is obviously a critical environmental factor in celiac disease. Whether other environmental factors are also involved is still an open question. Gut infections may be involved. Adenovirus 12 has been in the forefront among the candidate microorganisms. This virus was originally proposed as a candidate because of partial linear homology over 12 amino acids in a virus protein and an α gliadin (25). Based on our current understanding of the pathogenesis of celiac disease, the rationale for the candidacy of this virus is weak, and there are few epidemiologic data supporting a role for this virus (26).

CHARACTERISTICS OF THE CELIAC LESION

The lesion in celiac disease is localized in the proximal part of the small intestine. The full-blown lesion is characterized by villous atrophy, crypt cell hyperplasia, and leukocytic cell infiltration of the epithelium and the lamina propria. Intraepithelial lymphocytes (IELs) of both the TCRαβ+ CD8+ CD4− and the TCRγδ+ CD8− CD4− phenotypes are expanded in celiac disease. In contrast to the TCRαβ+ CD8+ IELs, which return to normal when gluten is removed from the diet, the TCRγδ+ IELs appear to remain at an increased level (27). Interestingly, most TCRγδ+ IELs express the Vδ1 TCR variable region (28,29). Spies and coworkers (30) have shown that γδ T cells expressing this variable region recognize MICA and MICB molecules—molecules that are expressed by intestinal epithelial cells (31). Activated human IELs are able to produce various cytokines including interferon-γ (IFN-γ), interleukin 2 (IL-2), IL-8, and tumor necrosis factor-α (TNF-α), and are known to have a lytic potential (32).

In the lamina propria, there is a marked infiltration of TCRαβ+ T cells in the active lesion. These T cells are mostly CD4+ and carry a memory phenotype (CD45RO+) (33). Lamina propria T cells actively produce cytokines, of which IFN-γ seems to be dominant (34,35). This observation is consistent with the finding that gluten-specific T cells isolated from celiac lesions produce large amounts of IFN-γ (36).

Just beneath the epithelium in the normal mucosa there are many macrophage/dendritic-like cells that stain positive for CD68 (37). It is conceivable that these cells are involved in sampling of luminal antigens. The expression of HLA class II, ICAM-1, and CD25 molecules is increased in these macrophage/dendritic-like cells, suggesting that they are activated in the disease state (38–40).

In the normal small intestine, there is a balance between extracellular matrix formation by stromal cells and extracellular matrix degradation mediated by matrix metalloproteinases (MMPs). It has been suggested that increased extracellular matrix degradation plays a role in the villous atrophy of celiac disease. This is supported by the finding of a decreased ratio of cells expressing collagen I and tissue inhibitor of metalloproteinase (TIMP)-1 messenger RNA (mRNA) to those expressing MMP-1 and MMP-3 mRNA in untreated celiac disease (41). Expression of MMP-1 and MMP-3 mRNA is mainly localized to subepithelial fibroblasts and macrophages. It is likely that the increased expression of metalloproteinases is related to activation of mucosal T cells.
PEPTIDE BINDING TO THE CELIAC DISEASE—ASSOCIATED DQ2 AND DQ8 MOLECULES

Both HLA class I and class II molecules bind peptides in a groove located in their membrane distal part (42). Stable binding is achieved by multiple hydrogen bonds between amino acids of HLA and peptide main chain atoms. There are many polymorphic variants of HLA molecules. Amino acid residues, which differ between the polymorphic variants, are clustered around the peptide binding site, where they contribute to the formation of specific binding pockets. Side chains of amino acids of the peptide (so-called anchor residues) fit into these pockets, and their interaction with HLA contributes to the binding of the peptide. The binding site of HLA class II molecules, in contrast to class I molecules, is open at both ends, allowing the bound peptides to protrude. The class II peptide ligands thus vary in length. The interactions with HLA mainly take place in a core region of nine residues. Within this region, side chains of amino acids in positions P1, P4, P6, P7, and P9 dock into pockets of the class II binding site. The chemistry and size of the various pockets vary between the different class II alleles, so that some amino acids are preferred and some are not.

DQ2 has a unique preference for binding peptides with negatively charged side chains at the three middle anchor positions (Fig. 2) (43–45). The binding motif of DQ8 is different from that of DQ2, but DQ8 also displays a preference for binding negatively charged residues at several positions (that is, P1, P4, and P9) (46–48). Hence, both the DQ2 and DQ8 molecules share a preference for negatively charged residues at some of their anchor positions.

![Diagram of peptide binding groove of HLA-DQ2](Available at www.AnnualReviews.org.)
The observation that gluten-reactive CD4+ TCRαβ T cells can be isolated and propagated from intestinal biopsies of patients with celiac disease has been instrumental in recent achievements (49). Strikingly, such T cells in patients carrying the DR3-DQ2 haplotype were found to recognize gluten fragments presented by the DQ2 molecule rather than by other HLA molecules of the patients (49,50). Both DR3-DQ2-positive and DR5-DQ7/DR7-DQ2–positive antigen-presenting cells (that is, those carrying the DQA1*05 and DQB1*02 genes in cis or in trans configuration) are able to present the gluten antigen to these patient T cells. Likewise, T cells isolated from small-intestine biopsies of DQ2-negative, DR4-DQ8–positive patients predominantly recognize gluten-derived peptides when presented by the DQ8 molecule (49,50). Taken together, these results allude to presentation of gluten peptides in the small intestine as the mechanism whereby DQ2 and DQ8 confer susceptibility to celiac disease. HLA molecules are also important for determining the repertoire of peripheral T cells during maturation in the thymus. A thymic effect of the same DQ molecules on the TCR repertoire selection is, however, not excluded by these results.

IMPORTANCE OF GLUTEN DEAMIDATION FOR T-CELL RECOGNITION

Wheat gluten is a mixture of a large number of gliadin and glutenin polypeptides. Generally, gluten proteins are rich in proline and glutamine residues, whereas many other amino acids—including glutamic acid and aspartic acid—are unusually rare. Proteins of the gliadin fraction can be subdivided according to their sequence into the α, γ, and ω gliadins (51). Initially it was difficult to reconcile the DQ2 (and DQ8) binding motifs with presentation of gluten peptides, because gluten proteins have an unusual scarcity of negatively charged residues.

A clue that might help explain this paradox came from the observation that the stimulatory capacity of gliadin preparations for gliadin-specific intestinal T cells was significantly enhanced following treatment at high temperatures and low pH (52). These conditions are known to cause nonspecific deamidation of glutamines to glutamic acid and may thus convert gliadin from a protein with very few peptides with the potential to bind to DQ2/DQ8 into one with many. An important and general role for deamidation of gluten for T-cell recognition was sustained by analysis of the response pattern of a panel of polyclonal, gliadin-specific T-cell lines derived from biopsies (53). All the cell lines responded poorly to a gliadin antigen prepared under conditions of minimal deamidation (chymotrypsin digestion) when compared with the same antigen that had been further heat-treated in an acidic environment.

The characterization of gluten epitopes recognized by intestinal T cells has extended our knowledge of the importance of deamidation for T-cell recognition. Of the epitopes characterized up to now, most (DQ2-γ-gliadin-I, DQ2-α-gliadin-I, and DQ2-α-gliadin-II) fail to stimulate T cells in their native form, but are potent antigens when
a single glutamine residue is exchanged with glutamic acid in certain positions. For one DQ8-restricted epitope (DQ8-α-gliadin-I), the T-cell recognition is augmented by introduction of negatively charged residues (54), whereas this is not seen for another DQ8-restricted epitope (DQ8-glutenin-I) (55). These data show that most, but not all, gluten-specific intestinal T cells from patients with celiac disease recognize gluten proteins only after they have undergone deamidation.

TISSUE TRANSGLUTAMINASE DEAMIDATES GLUTEN PEPTIDES

There is accumulating evidence that deamidation in vivo is mediated by the enzyme tTG (56,57). tTG is expressed in many different tissues and organs. In the small intestine, it is mainly expressed just beneath the epithelium in the gut wall (56). The activity of tTG in the small-intestine mucosa in patients with untreated celiac disease is increased compared with controls (58). The enzyme is present both intracellularly and extracellularly, and in the extracellular environment, tTG has been shown to play a role in extracellular matrix assembly, cell adhesion, and wound healing (59). The calcium-dependent transglutaminase activity of tTG catalyzes selective cross-linking or deamidation of protein-bound glutamine residues (60). In contrast to the nonenzymatically mediated deamidation that results in a near-random deamidation of the often numerous glutamine residues in gliadin peptides, tTG appears to carry out an ordered deamidation of some few specific glutamines.

In all the known major DQ2 and DQ8 restricted gluten epitopes recognized by gut T cells of adult patients, there are glutamic acid residues modified by tTG, which is important for T-cell recognition (53,54,61). Interestingly, the deamidation of glutamine residues that are not targeted by tTG (for example, by acid treatment) can be deleterious for T-cell recognition (57,62). This suggests that deamidation in vivo is mediated by tTG (Fig. 3). This idea is further supported by the results of experiments where T cell lines have been established from biopsies challenged with a minimally deamidated gliadin antigen (chymotrypsin-digested). In all but one of 18 adult patients, the established T-cell lines only barely responded to the chymotrypsin-digested gliadins, but efficiently recognized the in vitro tTG-treated variants of the same gliadins (63). Moreover, the addition of the tTG inhibitor cystamine during the gliadin challenge often resulted in T-cell lines with abolished or reduced responses to deamidated gliadin (63).

Normally, we do not mount immune responses to edible proteins. This phenomenon, which is termed oral tolerance, is believed to occur because of active tolerization toward edible proteins. In keeping with this thinking, oral tolerance to gluten in patients with celiac disease is either not established properly or disrupted. Given the preferential intestinal T-cell response to deamidated gluten fragments in patients with celiac disease, it may be that deamidation is central to the disturbance of oral gluten tolerance in such individuals. Deamidation increases the binding affinity of gliadin peptides for DQ2 from poor but significant binding to binding having reasonable but by no means exceptional affinity (53,61).
FIG. 3. CD4+ T lymphocytes in the lamina propria recognize deamidated gluten peptides presented by DQ2 or DQ8 molecules expressed by antigen-presenting cells (APC). The deamination in vivo is likely to be mediated by the enzyme tissue transglutaminase, which in the gut mainly is expressed beneath the epithelium.

FIG. 4. A cartoon showing the amino acid sequence (one-letter code) and binding to DQ2 of the three known gluten epitopes recognized by intestinal T cells of patients with celiac disease. Two of the epitopes stem from α gliadins (DQ2-α-gliadin-II and DQ2-α-gliadin-I) and one from γ gliadins (DQ2-γ-gliadin-I). The T cell recognition of all three epitopes requires glutamine (Q) to glutamic acid (E) conversions in certain positions. This can be mediated by the enzyme tissue transglutaminase. (From the Annual Review of Immunology, Vol 18, 2000). (Available at www.AnnualReviews.org.).
The moderate binding affinity of these epitopes concurs with the finding that they do not carry optimal anchors in all the anchor positions. It is interesting that the modified glutamine residues for the three defined DQ2-restricted gliadin epitopes recognized by intestinal T cells occupy different pockets within DQ2 (Fig. 4). This suggests that the altered affinity of the gliadin peptides for DQ2 is a critical factor involved in loss of tolerance rather than recognition of a single "pathogenic" motif that binds to DQ2.

GLUTEN T-CELL EPITOPEs IN CELIAC DISEASE: HOW MANY?

There are several epitopes in gluten that are recognized by small-intestine T cells of patients with celiac disease (52). Recent results from my laboratory (63a) and that of Koning in Leiden (63b) indicate that there may be more than ten distinct DQ2-restricted epitopes. The existence of multiple epitopes raises several interesting questions. Are only some of the epitopes pathogenic and thereby relevant to the HLA association? Are responses toward some of the epitopes generated during the early phases of disease development, whereas the responses to others are a result of epitope spreading? Are different epitopes recognized by distinct groups of patients (for example, children as opposed to adults)? Are some epitopes more relevant to disease because responses to them are found in most patients or because there is a higher precursor frequency of T cells in the lesion specific for these epitopes?

The answers to most of these questions must await further investigation. At present we know that for the DQ2-α-gliadin-I and DQ2-α-gliadin-II epitopes, intestinal T-cell reactivity is found in most, if not all adult DQ2+ patients (61), whereas for the DQ2-γ-gliadin-I epitope, intestinal T-cell reactivity is found in fewer DQ2+ patients (53). Less is known about the DQ8-restricted epitopes because few DQ8+ patients have been tested so far. However, the DQ8-α-gliadin-I appears to be commonly recognized (54). What causes the variance in responsiveness to the different epitopes and whether this reflects qualitative or quantitative differences between the patients is presently unclear.

GLUTEN-SPECIFIC T CELLS MAY PROVIDE HELP FOR AUTOANTIBODY PRODUCTION

The serum IgG and IgA antibodies to tTG (also termed antiendomysial antibodies) are a hallmark of celiac disease, and detection of serum IgA tTG antibodies is used to predict the disease (64,65). Dieterich et al. (3) showed that tTG/gliadin complexes could be formed in vitro and argued that these complexes may contain neoepitopes that trigger mucosal T cells and break tolerance. The production of the anti-tTG IgA antibodies must be dependent on cognate T cell help to facilitate the isotype switching of the autoreactive B cells. Dieterich suggested that the necessary help for the B-cell production of anti-tTG IgA could be provided by autoreactive T cells specific for tTG. The existence of tTG-reactive T cells is doubtful. As tTG is expressed in the thymic epithelium, it is likely that tTG-reactive T cells are deleted during negative
selection in the thymus (66). Moreover, the consequence of having autoreactive T cells specific for such a ubiquitously expressed enzyme as tTG would most likely be systemic autoimmunity.

As an alternative, tTG/gluten complexes may allow gluten-specific T cells to provide help to tTG-specific B cells by a mechanism analogous to the classical hapten carrier system (67). tTG-specific B cells will bind tTG/gluten complexes through their surface immunoglobulin. The complex will be processed, and gluten fragments may be produced that will bind to DQ2 or DQ8. Gluten-specific T cells may recognize this on the surface of the tTG-specific B cells and thereby provide cognate help for B cell maturation, isotype switching, and antibody secretion. This model can explain why tTG antibody levels in celiac disease are dependent on the presence of gliadin in the diet, as its removal will also abolish the T cell help needed for antibody production.

**MECHANISMS INVOLVED IN FORMATION OF THE CELIAC LESION**

The evidence discussed above provides strong evidence that CD4\(^+\) TCR\(\alpha\beta\)\(^+\) T cells in the lamina propria are central in controlling the immune response to gluten that produces the immunopathology of celiac disease (Fig. 3). The knowledge of the events downstream of T-cell activation is, however, still incomplete. Knowing that the immune system generally uses a multitude of effector mechanisms to fight its opponents, it is reasonable to assume that there may be multiple effector mechanisms involved in the creation of the celiac lesion. Adding to the complexity, recent in vitro organ culture studies have indicated that gluten exerts additional immune relevant effects that are independent of T cell activation (68,69). Some of these effects have rapid kinetics and conceivably the direct effects of gluten may facilitate subsequent T-cell responses.

Cytokines produced by lamina propria CD4\(^+\) T cells may be involved in the increased crypt cell proliferation and the increased loss of epithelial cells. IFN-\(\gamma\) induces macrophages to produce TNF-\(\alpha\). TNF-\(\alpha\) activates stromal cells to produce keratinocyte growth factor (KGF), and KGF causes epithelial proliferation and crypt cell hyperplasia (70). IFN-\(\gamma\) and TNF-\(\alpha\) can jointly have a direct cytotoxic effect on intestinal epithelial cells (71). It is also conceivable that IELs, and in particular \(\gamma\delta\) T cells, play a role in the epithelial cell destruction by recognizing MIC molecules induced by stress (30).

Alterations to the extracellular matrix can also distort the epithelial arrangement, because the extracellular matrix provides the scaffold on which the epithelium lies. Enterocytes adhere to basement membrane through extracellular matrix receptors, so that modification or loss of the basement membrane can result in enterocyte shedding. There is evidence for increased extracellular matrix degeneration in celiac disease, and this may be an important mechanism for the mucosal transformation found in the disease (41). The increased production of metalloproteinases by subepithelial fibroblasts and macrophages is likely to be directly or indirectly induced by cytokines that are released from activated T cells.
We do not yet know whether the autoantibodies play a role in the pathogenesis of celiac disease. The tTG antibodies can inhibit the activity of tTG. This can cause villous atrophy by blocking interactions between mesenchymal cells and epithelial cells during the migration of epithelial cells and fibroblasts from the crypts to the tips of the villi (72). Moreover, the tTG antibodies may modulate the deamidating activity of tTG, in an inhibiting or a promoting fashion (73). Further research will tell us what role these antibodies play.

TRANSLATION OF THE NEW KNOWLEDGE INTO TREATMENT

The increasing insight into the molecular and cellular basis of celiac disease should yield benefit for patients. Knowledge about which gluten epitopes are recognized by gut T cells should allow improvements to the methods whereby gluten-free foods are assessed. Moreover, the new knowledge should uncover novel targets for treatment. There are already some attractive possibilities. Activation of CD4+ gluten-specific T cells appears to be a critical checkpoint in the development of celiac disease, and interference with this step in the pathogenesis should be an effective way of controlling the disease. One possibility, which is basically an extension of today’s treatment with a gluten-free diet, is to produce wheat seeds that are devoid of T-cell epitopes, either by breeding programs or transgenic technology.

tTG is a target for intervention because of the critical role it plays in generating gluten T-cell epitopes. Inhibitors of tTG activity exist and likely inhibitors suitable for use as drugs can be developed. The biggest problem with this approach is that tTG inhibitors may have unacceptable side effects. tTG is involved in many different physiologic processes including programmed cell death (59).

Another strategy would be to aim directly at the gluten-specific T cells. If patients with celiac disease have a normal oral tolerance to native gluten proteins but a disturbed tolerance to deamidated gluten peptides, exposing the gut immune system to deamidated peptides may establish oral tolerance to the deamidated gluten peptides as well. This approach would use the body’s own mechanism to silence T cells. Alternatively, one could try to silence gluten-specific T cells directly by using soluble dimers of HLA/peptide complexes, which have been shown to induce antigen-specific apoptosis because of inappropriate T cell stimulation (74).

The central role of DQ2 and DQ8 in presenting gluten peptides offers yet another target for intervention. Blocking the binding sites of these HLA molecules would prevent presentation of disease-inducing gluten peptides. The challenge with this approach will be to find an efficient way of targeting and blocking the binding sites of DQ molecules, which are continuously synthesized by antigen-presenting cells. This approach—the blocking of peptide presentation—has also been suggested as treatment for other HLA-associated diseases. Celiac disease should be better suited to this approach because drug delivery in the gut is easy compared with, for example, the joints in rheumatoid arthritis or the pancreas in type 1 diabetes.

Whatever new therapeutic methods are introduced in celiac disease, they will have to be shown to be better than the current gluten-free diet regime. Long-term safety
must also be taken into account when devising any new treatment. Although there are already interesting therapeutic principles with a good, rational basis that can be tested, it may for this reason take some years before any new treatments become a reality.

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REFERENCES


DISCUSSION

Dr. Belli: What molecular evidence do we have for oat tolerance in celiac disease?

Dr. Sollid: For the time being the evidence is clinical. There are ongoing trials in Finland in which patients with celiac disease and dermatitis herpetiformis have been receiving oats for up to 5 years now, and there are no signs of immune activation or changes in mucosal architecture (1). What the long-term effects will be in terms of protection against osteoporosis and infertility and those more difficult endpoints is very hard to say, but I think the general understanding by scientists is that oats are tolerated. We now need to see whether we can find T-cell reactivity to any oats protein, as an independent line of evidence in support of oats being harmless.

Dr. B. Koletzko: You made a convincing case for HLA-DQ2 as providing the key to antigen presentation. My understanding was that the presence of the DR3 or DR5/7 genotype is really an indicator of increased risk but not an essential prerequisite for celiac disease. If that is so, are there other mechanisms involved in parallel to the antigen presentation by HLA-DQ2? Could there be a variety of pathophysiologies?

Dr. Sollid: I think this is the best argument for saying that there are non-HLA genes that contribute. Of the total genetic risk, HLA contributes perhaps as much as 30% to 50% and the rest of the effect is conferred by non-HLA genes, but the effect of each of these genes is modest—so small that they are hard to find by a genome-wide screen, where the power is relatively low. It could also be the case that different patients have different susceptibility genes, with disease heterogeneity. The chances of finding such genes then become much smaller. Despite the relatively gloomy data on the evidence of the non-HLA genes, I'm convinced that they exist, but we don't have the tools to locate them at this point.

Dr. B. Koletzko: My second question relates to the recommendations of Swedish pediatricians that gluten should be introduced earlier in the diet at the time of breastfeeding, based on a plausible argument that this would reduce the incidence of celiac disease (2). When one studies the data, however, there is an anomaly. It is apparent that while the recommendations of the Swedish pediatricians dated from 1996, the peak incidence of celiac disease was reported to be in 1994, and had declined by more than half by the time the recommendations were issued. Obviously one would expect that between the application of a new recommendation, if it were...
effective, and any effect on disease incidence, there would be a lag period, so it seems as if the disease prevalence anticipated the wisdom of the Swedish pediatricians. There does not seem to be a good case for a causal relation.

**Dr. Sollid:** I agree entirely. My Swedish colleagues say that there was a huge debate in Sweden on this topic, including several front-page articles in major newspapers on the subject before the national recommendations were changed. When the gluten consumption of these infants was measured, it was apparent that there was decline in the amount consumed that occurred before the change in the national recommendations. This could be due to the public debate. But the data have also been used to argue for a role of breastfeeding in preventing celiac disease. The duration of the breastfeeding has been increasing over the years with the decline in celiac disease incidence, and from an immunologic point of view, there may be good reason to believe that breastfeeding is implicated in protection from development of celiac disease. Our model involves the creation of an inflammatory environment. We think the presentation of deamidated peptides will only occur if you already have inflammation, and such inflammation can be caused by an infection. Breastfeeding would protect you from that. So that could explain why you don't end up in a situation where you start to create dangerous peptides.

**Dr. B. Koletzko:** I certainly follow the argument that breastfeeding may be protective, but it doesn't really match the epidemiology. The reported change in the incidence rate does not parallel the change in breastfeeding habits.

**Dr. Sollid:** I agree. I think that the amount of gluten intake correlates much better than the change in the national recommendation, and this mostly reflects the fact that parents started to think for themselves. There was a major debate on this in Sweden.

**Dr. Marquardt:** I did understand the role of transglutaminase in the generation of peptides that then trigger the immune response, but why does one make antibodies against tissue transglutaminase?

**Dr. Sollid:** The basic answer is, we don't know. Tissue transglutaminase is expressed in almost all organs in the body, including the thymus, and I don't think there are any tissue-transglutaminase-specific T cells, so it's also very hard to see a role of gluten in this model. Tissue transglutaminase can cross-link proteins and a German group showed that it can cross-link glutamine residues of gluten proteins onto lysine, so you form a complex (3). Immunologists recognize this as a hapten-carrier complex. This complex is taken up by the B cell, which processes the antigens, and you end up with the gluten peptide bound to the class II molecule. We know that there are T cells specific for gluten peptide, and so this shortcut of the immune system can fool it into creating antibodies to self-protein. If you take away gluten from the diet, you basically take away this peptide. Although we don't have experimental data to prove this, I think it's the simplest model you can invoke to explain how antibodies to self antigens are driven by a T cell response to an exogenous antigen. I believe this is also relevant to other autoimmune diseases.

**Dr. Fowler:** I'm interested in the physiologic role of transglutaminase. Could you enlarge on that?

**Dr. Sollid:** Tissue transglutaminase is categorized as a wound-healing enzyme. If you induce a wound experimentally, you increase its expression at least ten-fold, and in the active celiac lesion, you also have increased expression. One problem we do not understand is what dictates cross-linking and what dictates deamidation. What our model requires is deamidation, and people who have worked with the biology of tissue transglutaminase say that the primary substrate for this enzyme is primary amines—lysine, spermidine, or spermine—and if those are present, you won't get deamidation. I would look at it from the other viewpoint. T cells basically record what is present, and what T cells recognize is the introduction of deamidated residues, which are very specific and which concur with the specificity of the enzyme. We do
not really understand the microenvironment of the gut where these epitopes are formed. It could be that you deplete the storage of these primary amines when you have breakage of the epithelial barrier. You flood the lamina propria with gluten proteins and consume the primary amines, and you then start to create these epitopes. If you have a situation where you have more gluten, more substrate, and a higher enzyme level, you will reach that situation more rapidly.

To try to mimic what is going on in this complex system in the gut is very difficult with ex vivo testing. We don’t really know whether these peptides need to be taken up by the antigen-presenting cells and whether they can bind at the surface.

Dr. S. Koletzko: A couple of years ago a large epidemiologic study from Italy was published in *Gastroenterology* on more than 900 celiac patients diagnosed during childhood or adult life. The investigators showed that the earlier celiac disease was diagnosed and treated, the less problem there was with other autoimmune diseases later on. They showed a clear dose-response effect. Could you comment on that report? Do you believe you can prevent other autoimmune diseases by treating celiac disease early in life?

Dr. Sollid: What is clear is that there is an overrepresentation of many autoimmune diseases in celiac disease—for example type 1 diabetes, thyroiditis, and some other diseases. The Italian investigators have not convinced me that celiac disease predisposes to the development of other autoimmune diseases. If you follow a cohort of patients with celiac disease, you will have an accumulation of other autoimmune diseases regardless of whether or not a gluten-free diet is in place. To prove that there is a difference, I think you would need larger numbers than were presented in that Italian study. However, this is not my field of expertise.

REFERENCES

