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Innate and adaptive immunity: the Yin and Yang of celiac disease

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Summary: Celiac disease is a multigenetic complex inflammatory disorder with an autoimmune component, induced by gluten, a protein found in wheat. It is a unique human disease model to dissect the innate and adaptive immune mechanisms underlying T-cell-mediated tissue destruction and the development of T-cell lymphoma in conditions of chronic T-cell activation.

Introduction

Celiac disease is a T-cell-mediated immune disease and is found mainly in individuals of Caucasian ancestry. It results in the destruction of the surface epithelium and flattening of the mucosa. Celiac disease is triggered in genetically susceptible individuals by wheat grain storage proteins, collectively known as gluten proteins. This lifetime disease can be treated by a diet excluding gluten. Enteropathy (celiac)-associated T-cell lymphomas (EATLs) constitute a major distinctive but rare complication of celiac disease. Celiac disease is a unique model to dissect the innate and adaptive immune mechanisms underlying tissue destruction and to study how chronic T-cell activation can lead to the development of lymphoma.

Some highlights in celiac history

Samule Gee was the first to clearly define celiac disease and to recognize the role of diet. In 1888 he stated, 'The allowance of farinaceous foods must be small, but if the patient can be cured at all, it must be by means of diet.' Sixty years later, the Dutch pediatrician Willem Karel Dicke suggested a direct role for gluten and described the histological intestinal alterations in celiac disease (reviewed in 1). In the 1980s, the evidence for a primary association of celiac disease with particular DQ molecules was described (reviewed in 2). This finding provided an explanation as to why divergent DR associations were observed in different Caucasian populations. This observation was followed by the identification of intestinal

$CD4^+$ T-cell clones recognizing gliadin peptides presented by DQ2 or DQ8 molecules (3, 4). Around the same time, it was found that celiac patients develop antibodies to gliadin and an unknown component of endomysium. In 1997, Dieterich and colleagues (5) discovered that anti-endomysium antibodies were in fact directed against an autoantigen, tissue transglutaminase (TG), and that gluten constituted an excellent substrate for this enzyme. The impact of TG on the anti-gliadin $CD4^+$ T-cell response was demonstrated by the finding that intestinal gliadin-restricted $CD4^+$ T cells preferentially recognized gluten peptides deamidated by TG (6, 7).

The establishment of a molecular basis for the adaptive anti-gliadin immune response has pushed aside the idea that gluten could have a direct 'toxic' (innate) effect on the intestinal mucosa, in particular on intestinal epithelial cells (IECs) (8–11). At the time when celiac disease began to be studied, the concept of innate immunity was not developed. Therefore, the terminologies used to define the effects of gluten were ambiguous. In other words, no distinction was initially made between the innate and adaptive immune effects of gluten. However, quite early in the history of celiac disease, a number of investigators recognized the immediate effects of gluten on the epithelium, and even more importantly, they identified a sequence in α-gliadin (peptide 31–49 or 31–43) (12–16) that induced epithelial alterations in the absence of $CD4^+$ T-cell activation.

In contrast to $CD4^+$ T cells, intraepithelial $CD8^+$ T lymphocytes (IELs) were for a long time viewed as playing no role, or at best a secondary role, in the pathogenesis of celiac disease. Yet, the observations that IELs could undergo malignant transformation and express high levels of cytotoxic granules and interferon-γ (IFN-γ) indicated that IELs were activated (reviewed in 17). As the molecular mechanisms underlying epithelial cell killing by IELs were identified, the role of IELs in the pathogenesis of celiac disease became recognized (16, 18, 19). In parallel, the concept emerged that uncontrolled expression of the cytokine interleukin-15 (IL-15) played an important role in the activation and malignant transformation of IELs (18, 20, 21).

Clinical presentation and epidemiology

Studies, based on serologic screening, have revealed that celiac disease occurs in adults and children at rates approaching 1% (reviewed in 17). It is, therefore, considered as one of most common chronic diseases. Celiac disease can be diagnosed at any age. Infants and young children present with diarrhea, abdominal distension, and failure to thrive. However, vomiting, irritability, anorexia, and constipation are also common. Older children often present with extraintestinal manifestations, such as short stature, neurologic symptoms, or anemia (reviewed in 17). The mean age of diagnosis in adults is between 40 and 50 years of age. Women are diagnosed at two to three times the rate of men, though this gender predominance is lost in older individuals. The most frequent single mode of presentation in adults is diarrhea, though this mode of presentation accounts for less than 50% of cases. Other presentations include serological presentations with the presence of anti-TG and/or anti-endomysium antibodies in the absence of clinical symptoms, iron deficiency, osteoporosis, neurological symptoms, and dermatitis herpetiformis.

The duodenal biopsy is currently the gold standard in the diagnosis of celiac disease. Patients typically come to biopsy because of the result of positive serologic tests for celiac disease. Intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy are required for the consideration of the diagnosis. Finally, the diagnosis is not established until there is an unequivocal response to gluten withdrawal, symptomatic and histologic. Marsh (22) described the characteristic pathologic findings as a continuum. The initial lesion (Marsh 1) consists of an intraepithelial lymphocytosis in normal appearing villi (lymphocytic enteritis). The Marsh 11 lesion has crypt hyperplasia in addition to the intraepithelial lymphocytosis. The majority of those diagnosed with celiac disease have a Marsh 111 lesion with villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis. The Marsh 111 lesion includes partial, subtotal, and total villous atrophy. The histologic findings are characteristic but not specific for celiac disease. Lymphocytic enteritis may be seen in autoimmune diseases and *Helicobacter pylori* infection, and the Marsh 1 and 111 lesions may be seen in tropical sprue, human immunodeficiency virus enteropathy, and giardiasis.

The most sensitive and specific blood tests are the immunoglobulin A (IgA) subtypes of the anti-TG and endomysial antibodies. The sensitivity and specificity are $> 90\%$, though the titers can be very low in the presence of mild villous atrophy (23, 24). Anti-gliadin antibodies, in particular IgG anti-gliadin antibodies, can be found in normal individuals and are therefore not specific enough to be used in diagnosis (25).

Autoimmune diseases occur 3–10 times more frequently in celiac patients than in the general population (26, 27). The range of associated autoimmune diseases is broad (reviewed in 17). They include type 1 diabetes, primary biliary cirrhosis, Sjögren's syndrome, and peripheral neuropathy. Malignancies are also associated with celiac disease (reviewed in 17, 28). They comprise squamous carcinoma of the oropharynx and

esophagus, adenocarcinoma of the small intestine, as well as non-Hodgkin's lymphoma. The lymphomas are both T- and B-cell types and occur at intestinal and extraintestinal sites. The risk of developing a lymphoma is considered today to be two- to fourfold higher in celiac patients than in the control population (29).

The possibility of an association between malabsorption and lymphoma was raised for the first time in 1937 (30). Fifty years later, the term enteropathy-associated T-cell lymphoma was given by O'Farelly (31). Finally, the intraepithelial T-cell origin of this lymphoma was demonstrated using an antibody directed against the integrin aEβ7 expressed by IELs (32, 33). EATL is typically not found in other inflammatory and autoimmune intestinal disorders, and it constitutes a unique complication of celiac disease.

Did genetics say all that it had to say?

Celiac disease is a multigenetic disorder. The high prevalence rate of 10% among first-degree relatives (34–36) and a concordance rate in monozygotic twins of 75% (37) indicate the primordial role of genetics in the susceptibility to develop celiac disease. The human leukocyte antigen (HLA) region is thought to confer 40% of the genetic risk, the rest of the genetic risk being attributable to yet undefined non-HLA genes (reviewed in 2).

Genetic risk conferred by the HLA locus

The HLA locus is part of a conserved extended haplotype region that includes the major histocompatibility complex (MHC) class II, class III, and class I regions, and adjacent loci. This region is highly polymorphic, and genetic associations within this region are difficult to define because of the issues related to linkage disequilibrium. Nonetheless, there is strong genetic and functional evidence today that the primary association is with DQ2 (DQA1*05/DQB1*02) and, to a lesser degree, with DQ8 (DQA1*0301/DQB1*0302) (reviewed in 2). Koning and colleagues (38) propose that the capacity of the HLA-DQ2 molecule DQA1*0501/DQB1*0201 to bind a broader range of gluten peptides than the HLA-DQ2 molecule DQA1*0201/DQB1*0202 may explain why the HLA-DR3DQ2, but not the HLA-DR7 DQ2 haplotype, confers a high risk to develop celiac disease. In addition, several studies suggest that carrying double dose of the predisposing DQ molecules increases significantly the probability of developing celiac disease (38, 39).

The strong association with DQ2 and DQ8 molecules does not preclude the possibility that other HLA genes could modulate the anti-gliadin immune response and have an impact on the pathogenesis of celiac disease. For instance, the MICB*10 gene, coding for MHC class I chain-related gene B (MICB) molecules, was found to be associated with celiac disease (40, 41). Functional studies further supported the potential role of MICB in celiac disease by demonstrating its upregulation in active celiac patients (16, 19) and its induction on epithelial cells upon gluten challenge (16, 42). There is also evidence for an association with TNF2 encoded within the MHC class III region (43, 44). Finally, the question as to why IgA deficiency and common variable immunodeficiency patients have a higher incidence of celiac disease and/or alterations of their intestinal epithelium remains open (45, 46). One potential explanation would be that this patient population classically carries the A1-B8-DR3 haplotype and hence frequently expresses the DQ2 molecule. However, it remains possible that HLA non-MHC class II genes cause epithelial cell damage in these patients, independent of intestinal infections.

Genetic risk conferred by non-HLA genes

There is strong support today for the role of non-HLA genes in celiac disease. A small controversial effect of the CTLA-4 gene, which encodes a molecule involved in T-cell inhibition, has been reported (47–51). Evidence exists for a strong linkage at 5p31–33 (52–54) and also, albeit to a lesser degree, at 19p13.1 (55), 9p 21 (55), and 11q (53, 56). In parallel to linkage studies, several investigators have tried to identify new genes through a candidate gene approach without much success. Because of our current knowledge pointing to the role of innate immunity and epithelial cells in the pathogenesis of celiac disease, the search for specific epithelial genes and genes coding for innate molecules may be of interest.

Is celiac disease an inflammatory, autoimmune, or allergic disorder?

The boundaries between inflammatory, autoimmune, and allergic diseases are difficult to draw. Celiac disease is not a food allergy, because unlike peanut and milk protein allergy, it does not involve IgE antibodies and mastocyte/basophil degranulation (reviewed in 57). However, anaphylactic reactions to gluten have been described, including in celiac patients. Celiac disease could be viewed as an inflammatory intestinal disease, because it is associated with an inflammatory infiltrate and high levels of IFN-γ expression in the intestinal mucosa (reviewed in 58). However, in inflammatory diseases, tissue cells are not typically the direct targets of a

T-cell-mediated immune response, and autoantibodies are absent. In this sense, the immunological presentation of celiac disease is much more reminiscent of the presentation of organ-specific autoimmune diseases such as diabetes. Thus, if the triggering factor, gluten, was not known, celiac disease would probably be viewed as a typical organ-specific autoimmune disease.

What is so special about gluten that challenges our immune system?

Humans have been cultivating wheat for about 10 000 years, although wild wheat types were occasionally harvested from thick stands during the hunter-gatherer phase of human evolution that preceded the development of agriculture. Considering that key elements of the adaptive immune system, such as B cells and T cells, had developed more than 400 million years ago in complex organisms living in the seas before sea-living organisms invaded the land, it appears that human's encounter with wheat through intensive cultivation has been relatively brief in evolutionary time.

Celiac disease is triggered in susceptible individuals by wheat grain storage proteins, collectively known as gluten proteins. They are cohesive with one another in wheat flour-water dough, such that kneading the dough under water or a stream of water dislodges starch granules from the starch-protein matrix, leaving behind a cohesive, elastic ball of protein, which has traditionally been called 'gluten'. Rye and barley grains have proteins very close in amino acid sequence to gluten proteins of wheat and on that basis (and some testing) are also active in celiac disease. However, a gluten ball cannot be washed from rye or barley flour-water mixtures, and traditionally, the proteins of rye and barley have not been classified as gluten proteins. Nevertheless, the celiac disease community (consisting mainly of patients and physicians) has adopted the term 'gluten' for any proteins that are active in celiac disease.

Wheat, oat, rice, finger millet, and teff usually fall in the *Festucoideae* subfamily of the grass family (*Gramineae*) when these grains are classified taxonomically (*Fig.* 1).

The *Festucoideae* subfamily includes the *Triticeae* tribe, oat, rice, finger millet, and teff (*Fig.* 1). Celiac disease is only induced by wheat, rye, and barley, which belong to the *Triticeae* tribe. Interestingly, a small subset of celiac patients can become intolerant to oat, and it has been demonstrated that the avenin proteins, which make up only about 10% of the total proteins of oat, are responsible (59).

The grain storage proteins of the *Triticeae* are rich in the amino acids glutamine and proline (mainly around 30 mole percent and 15 mole percent, respectively) and are often classed as prolamins on this basis, but this composition, although important, is insufficient in itself to explain toxicity. Sequence is also of great importance, insofar as maize or corn proteins may be rich in glutamine and proline apparently without having toxicity. In contrast, oat avenin proteins are relatively low in proline (10 mole percent), yet have at least one short sequence that appears to be an active sequence similar to one found in gluten proteins (59).

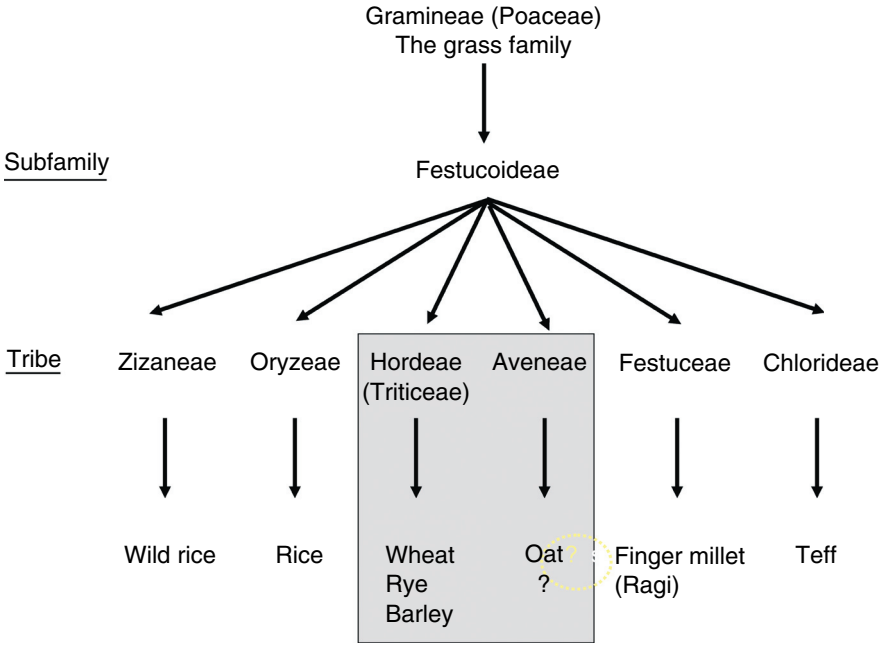

***Fig.* 1. Grains in the tribe *Triticeae* mediate celiac disease.** Species of interest in the *Festucoideae* subfamily of the grass family are depicted. It seems likely that only grains in the tribe *Triticeae* mediate celiac disease. There is some evidence indicating that a subset of celiac patients can become intolerant to oat, which shares some common sequences with gliadin.

The gluten proteins have been classified first into two major solubility fractions, the gliadins (monomeric proteins) and glutenins (polymeric forms of prolamins in which the protein subunits are linked to one another by disulfide bonds to form chains of protein subunits having a distribution of lengths). The glutenin fraction is mainly responsible for the elasticity of wheat flour dough and gluten itself. The gliadin proteins have next been classified into α-, β-, γ-, and ω-gliadins; these classifications are now recognized to have a basis in primary structure (sequence). The glutenin fraction is broken down into two main classes, the high-molecular-weight glutenin subunits and the low-molecular-weight glutenin subunits. All classes of gliadin and glutenin proteins are apparently harmful to celiac patients on the basis of *in vitro* and *in vivo* studies of the partially purified classes (9, 60). Because of the large numbers of prolines in their primary structures, which block cleavages of the polypeptide chains by digestive enzymes at positions immediately adjacent to the proline residues, all gliadins and glutenin subunits are likely to produce large slowly digesting or undigested peptides. Published studies have not been carried out to establish differences between classes, but similar amounts of proline in α-, β-, and γ-gliadins make it less likely that one class would produce peptides differing widely from another in size. Size of the residual peptides, during and after protein digestion in the gastrointestinal tract, however, may be important to activity in celiac disease (61). In the case of the ω-gliadins, the fragments resulting from digestion may be expected to be larger than those of the other classes because of the greater amounts of proline in the primary structures of the two subtypes of ω-gliadins (1D-coded and 1B-coded). Several reports indicate activity for ω-gliadins in celiac disease (9, 60, 62).

The gluten proteins are coded by at least 100 genes, and this number is probably a minimum. Although the exact number of toxic sequences in the gluten proteins is not established, it appears likely that there are many such sequences. The word toxic is an ambiguous word that is classically used in the celiac disease field. Two categories of 'toxic' gluten peptides have been identified. The first category comprises 'immunogenic' peptides that are part of the adaptive immune response. These peptides have been mainly identified in α-gliadins but can also be found in glutenin and γ-gliadin. The immunodominant α-gliadin 56–75 peptide (63, 64), encompassed in the 33-mer described by Shan *et al.* (61), is the most studied peptide in this category. The 33-mer α-gliadin peptide is thought to play a major role in celiac disease, because it is not digested by enzymes of the intestinal environment, is a good substrate for TG and is seen by a majority of $CD4^+$ T cells in adult HLA-DQ2 patients. Furthermore, an immunodominant peptide restricted by DQ8 has also been described (65). The second category comprises 'innate peptides' that are not recognized by $CD4^+$ T cells but induce an innate-like response in the epithelium and antigen-presenting cells (APCs). The most studied peptide in this category is the α-gliadin p31–43 (or p31–49 peptide) (12–16). The innate response induced by this peptide is comprised of the expression of IL-15 and non-classical MHC molecules such as MIC and HLA-E (16), which were shown to play an important role in IEL-mediated epithelial cell killing (16, 19) (*Fig.* 2). Because the toxic sequences are widely distributed, changing the composition of the gluten proteins by removing proline or by deleting toxic sequences will probably diminish the quality of bread made from such modified wheat.

When does an anti-gluten response translate into celiac disease? What defines celiac disease?

To answer the first question, it is important to remember that anti-gluten IgG antibodies are found relatively frequently in non-celiac individuals (25) and that anti-gluten $CD4^+$ T-cell clones can be isolated from peripheral blood lymphocytes of

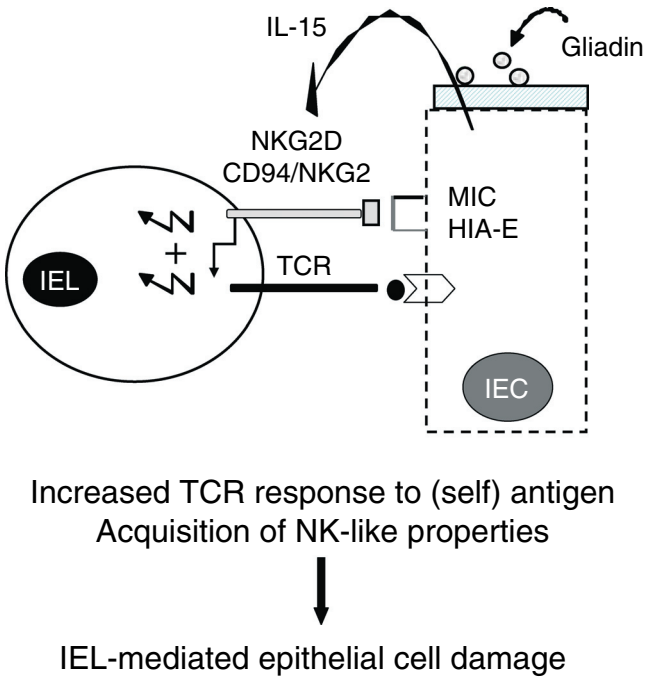

***Fig.* 2. Model accounting for epithelial cell killing by intraepithelial lymphocytes.** Gluten induces epithelial stress that results in the expression of interleukin-15 and non-classical class I molecules such as MIC and human leukocyte antigen-E. In turn, these molecules upregulate the expression of activating natural killer receptors (NKRs), which decrease the T-cell receptor activation threshold and confer NK-like properties.

normal individuals (66). These observations suggest that tolerance to gluten is easily broken and not synonymous with celiac disease. Whether the anti-gluten response seen in normal individuals takes place inside or outside of the gut is not known.

In contrast, the identification of IgA anti-gluten antibodies and even more so IgA anti-transglutaminase antibodies is viewed as a signature of celiac disease (reviewed in 17). The presence of IgA antibodies suggests that the anti-gluten immune response takes place in the gut. The presence of anti-transglutaminase antibodies suggests that gluten/TG2 complexes have been formed. Thus, the beginning of celiac disease could be defined as the development of an intestinal immune response against gluten/TG2 complexes (*Fig.* 3). What drives the formation of gluten/TG2 complexes remains to be defined, and whether or not the formation of such complexes plays a causal role in mucosal lesions remains to be determined.

Much like diabetes, the presence of autoantibodies and tissue infiltration by activated $CD4^+$ T cells is not sufficient to induce tissue destruction in celiac disease. The presence of anti-glutamic acid decarboxylase and anti-insulin antibodies without hyperglycemia is viewed as a pre-diabetic stage. Similarly, the presence of anti-TG2 antibodies in patients with normal histology could be viewed as a pre-celiac stage. Catassi and colleagues (67, 68) have described celiac disease as an iceberg in which patients with anti-TG (endomysium) antibodies and normal intestinal histology were defined as latent celiac patients. Some of these cases will progress and have histological lesions, whereas others will remain latent. What determines the passage of latent celiac disease to overt celiac disease remains an opened question. A hypothesis that we favor and will discuss later in more detail is that the arming of $CD8^+TCR\alpha\beta^+$ IELs to destroy stressed IECs determines the passage from latent to overt celiac disease (*Fig.* 3). Here again, a parallel with type 1 diabetes can be drawn, because it is well recognized that the activation of $CD8^+$ T cells, which recognize stressed β-islet pancreatic cells, is required for tissue destruction (69, 70).

Finally, borderline gluten-sensitive patients have been defined as patients with Marsh 1 lesion, i.e. increased IELs in the absence of epithelial cell destruction or other mucosal

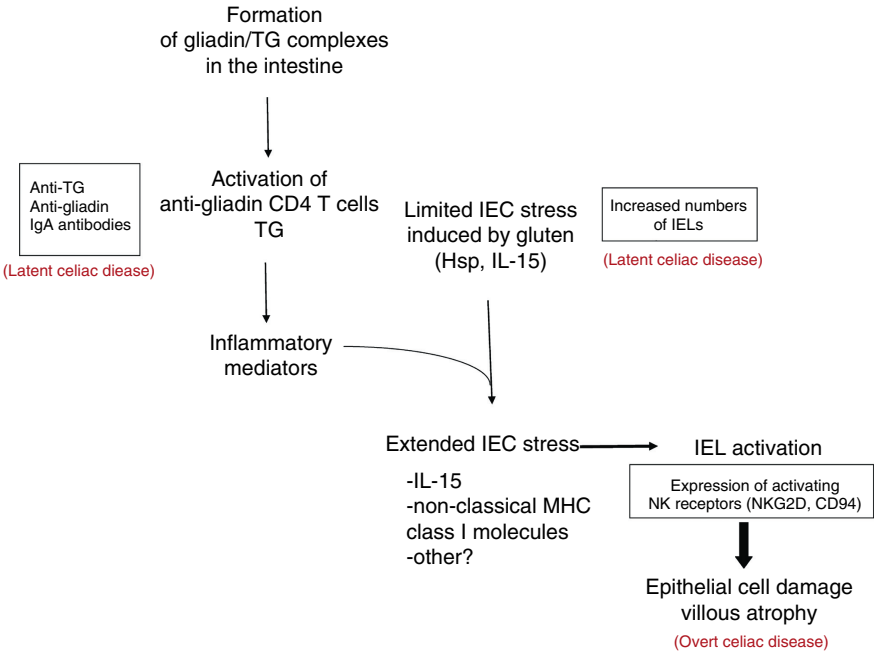

***Fig.* 3. Model accounting for the progression from latent to overt celiac disease and for the requirement of $CD4^+$ and $CD8^+$ T cells to produce epithelial damage.** Latent celiac disease can have three presentations: isolated IgA anti-TG and anti-gluten antibodies resulting from the formation of gluten/TG complexes, epithelial stress induced directly by gluten resulting in intraepithelial lymphocyte (IEL) infiltration, or a combination of the two presentations. Activation of $CD4^+$ T cells induces the liberation of inflammatory mediators that increase the level of epithelial stress and result in the expression of additional molecules including non-classical MHC class I molecules. At that stage, intestinal epithelial cells (IECs) express the whole panoply of molecules required for the upregulation of activating natural killer receptors (NKRs) on IELs, which decrease the T-cell receptor activation threshold and confer NK-like properties. In turn, NK-IELs kill stressed epithelial cells expressing the appropriate ligands for their NKRs.

alterations. Such intraepithelial infiltrations can be found in the absence of anti-TG2 or anti-endomysium antibodies. In a subset of these patients, gluten challenge can result in histological alterations. Conversely, gluten-free diet can result in the normalization of the number of IELs (71). We propose that an abnormal innate response to gluten resulting in epithelial stress and increased IL-15 expression may take place in these patients and be responsible for IEL expansion (*Fig.* 3).

Would celiac disease be celiac disease without TG?

The formation of gluten/TG complexes constitutes a key step in celiac disease (*Fig.* 3). They may be responsible for the induction of anti-TG antibodies by allowing gliadin $CD4^+$ T cells to provide help to autoreactive anti-TG B cells (72). Furthermore, because TG activity is increased in celiac patients (73), gliadin peptides are modified by a process called deamidation. This drives intestinal $CD4^+$ T cells to recognize preferentially deamidated peptides (7, 74). It is therefore unconcealed that TG impacts on the presentation of celiac disease. However, whether TG plays a causal role in celiac disease remains to be demonstrated.

Generalities on transglutaminases (reviewed in 75, 76)

Nine TG genes have been identified, eight of which encode active enzymes. The TG family comprises TG1, TG3, and TG5 expressed mostly in epithelial cells, TG2 which is expressed in most tissue, TG4 present in the prostate gland, factor XIII found in the blood, TG6 and TG7, whose tissue function and tissue distribution are unknown, and a protein that serves to maintain erythrocyte integrity. TGs require the binding of Ca^{2+} for their activity at supraphysiological concentrations. Furthermore, they are regulated by numerous post-translational modifications, such as phosphorylation and proteolytic cleavage. Finally, TGs are very short-lived proteins with a half-life around 11 h for TG2, and little is known about the regulation of their turnover. Thus, overall TG activity is highly regulated. TGs are virtually inactive under normal conditions and become active following cellular and tissue insult. The most important function of TGs is to catalyze post-translational modification of proteins by transamidation or deamidation. Transamidation results in the cross-linking of proteins by the formation of isopeptide linkages through acyl-transfer reactions, involving peptidyl glutamine residues as acyl donors and a variety of primary amines as acyl acceptors. Deamidation occurs in the absence of suitable amines involves water and occurs at lower pH. It results in the transformation of glutamine into negatively charged glutamate residues. TGs have profound and diverse biological effects. They modify numerous endogenous and exogenous proteins. They have been implicated in wound healing, blood clotting, cell differentiation, cell death, cell proliferation, and receptor-mediated endocytosis. They have also been implicated in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis and osteoarthritis. In addition, they are thought to be involved in chronic neurodegenerative diseases such as Alzheimer's disease and Huntington's disease. Finally, anti-TG antibodies are found in a number of autoimmune diseases such as type 1 diabetes, thyroid diseases, systemic lupus erythematosus, and Sjögren's syndrome, in which they may play a pathogenic role. Interestingly, a direct causal role for anti-TG3 antibodies has been proposed in dermatitis herpetiformis (75, 76).

TG2 shapes the anti-gliadin $CD4^+$ T-cell response

Gliadin is an excellent TG2 substrate because of its high glutamine (30–50 mole percent) and proline (15 mole percent) content. Koning and colleagues (65) have shown that the spacing between glutamines and proline determines which glutamines in gluten will be modified in the process of deamidation.

TG2 activity increases with inflammation and during the course of celiac disease; therefore, it is probable that the amount of gliadin peptides modified by TG2 increases over time, until deamidated gliadin peptides constitute the dominant form of gliadin peptides available in the intestinal environment. Such a scenario is compatible with the finding that $CD4^+$ T cells recognizing native (non-deamidated) gliadin peptides are more frequently found in children (77). Furthermore, the identification of $CD4^+$ T cells recognizing native gliadin peptides in blood and intestine indicates that deamidation is not required for the initiation of an anti-gliadin immune response (74, 77). Deamidation, instead, may play an important role in favoring the binding of gliadin peptides to DQ2 or DQ8 molecules, which possess binding pockets that have a preference for negatively charged amino acid residues (reviewed in 58, 78). It has been indeed shown that DR molecules can bind gliadin peptides (79) and that DR-restricted immune responses are predominantly found in the course of intestinal viral infections (80). Presently, we favor a model in which deamidation plays a role, not in allowing an anti-gluten response to take place but by altering the nature of the anti-gliadin response. Deamidation may reshape the anti-gliadin response by favoring the binding to DQ molecules and by amplifying the immune response. We have evidence in a

DQ8 transgenic (Tg) mouse model that native (non-deamidated) peptides are immunogenic, but $CD4^+$ T cells recognizing these peptides have a highly restricted T-cell receptor (TCR) repertoire (manuscript in preparation). Intriguingly, deamidation had a profound effect on the nature of the immune response by dramatically expanding the gliadin-reactive TCR repertoire (manuscript in preparation). Further studies are warranted to confirm and extend these findings to DQ2 and determine the exact impact of TG on the anti-gliadin CD4 T-cell response.

Other potential impacts of TG2 on the pathogenesis of celiac disease (reviewed in 75, 76)

TG may play a direct role by altering the activity and function of molecules involved in inflammation. For instance, TG2 has been shown to be involved in cell death and to increase phospholipase A2 activity and hence may enhance intestinal inflammation. Inflammation could also be promoted through the formation of histamine/gliadin conjugates that contribute to the mucosal IFN-γ response in celiac disease (80). TG may also play an indirect role in inflammation by forming TG2/protein complexes that function as hapten/carrier complexes, favoring antibody formation against TG2 substrates such as histone H2B and collagen. Finally, IgA anti-TG2 antibodies may play a role through the formation of immune complexes that activate the complement system and bind activating Fc receptors expressed by macrophages and dendritic cells. Thus, even though anti-TG antibodies are not the primary cause of celiac disease, they may indirectly contribute to tissue damage by promoting inflammation.

What is the role of the $CD4^+$ anti-gluten T-cell response?

The strong genetic link to DQ2 or DQ8 molecules along with the identification of gliadin DQ2- or DQ8-restricted $CD4^+$ T cells is the strongest argument in favor of a role of $CD4^+$ T cells in the pathogenesis of celiac disease. However, it is also clear that the activation of $CD4^+$ T cells is not sufficient to explain the destruction of surface epithelial cells, the dramatic increase in IELs, and their malignant transformation. All these characteristic features of celiac disease are not found in other intestinal diseases, such as Crohn's disease and autoimmune enteropathies, that are associated with $CD4^+$ T-cell activation and high levels if IFN-γ expression in the *lamina propria*. We favor a model in which IELs are the epithelial cell killers and $CD4^+$ T cells play a role in helping to arm the IEL effector response (*Fig.* 3). $CD4^+$ T cells could exert this function by releasing inflammatory mediators and hence creating a highly inflammatory environment, which would render epithelial cells more sensitive to the innate effects of gluten and impact on the activation status of IELs. The finding that the p31–43 αgliadin peptide, which is not recognized by $CD4^+$ T cells, can only induce epithelial cell alterations in celiac patients under gluten-free diet and not in controls (15, 16) can be explained in two ways that are not mutually exclusive. Either epithelial cells and APCs in celiac patients are uniquely sensitive to gluten or the inflammation that persists at some levels even under a gluten-free diet (the mucosa never returns completely to normal under gluten-free diet) is necessary for gluten to exert its innate effects (see below).

What do intraepithelial $CD8^+$ TCRαβT cells see and do?

Role of natural killer (NK) receptors in the regulation of tissue effector T cells and their role in autoimmunity

NKG2D and CD94/NKG2 NK lineage receptors are prominently expressed in tissues by effector cytotoxic T cells (CTLs) (18, 81–85). By recognizing conserved MHC-like ligands induced on tissue cells by stress, inflammation, and transformation, these NK receptors up- or downmodulate TCR stimulation, thus linking innate with adaptive immunity (reviewed in 86–88). This new layer of T-cell regulation at the effector stage serves to focus adaptive effector functions on infected or transformed tissues. Increasing evidence point to dysregulation of these mechanisms in autoimmune and inflammatory disorders (16, 18, 19, 70, 82, 83).

Role of IELs in celiac mucosal lesions

For many years, the role of IELs in the pathogenesis of celiac disease was disregarded, because no gluten MHC class I-restricted IEL could be identified. However, IEL activation in celiac disease was supported by the increase in IFN-γ^+ (89) and granzyme$^+$ IELs (90), and the malignant transformation of IELs leading to the development of EATL (91). Recently, this paradox was solved by the finding that epithelial cell killing was not driven by the recognition of gliadin peptides but by the recognition of stress molecules induced on epithelial cells in response to gluten (16, 18, 19) (*Fig.* 2). Solid experimental evidence now backs the following model that we proposed (84, 92). Gluten induces epithelial cell stress that results in the upregulation of IL-15 and non-classical MHC class I molecules. In turn, these molecules upregulate the activating NK receptors NKG2, which reduce the TCR activation threshold and mediate direct killing of epithelial cells expressing the appropriate non-classical MHC class I ligands

(HLA-E for CD94/NKG2 and MIC for NKG2D). The reduction in TCR activation threshold can lead IELs to respond abnormally to the intestinal flora or to low-affinity antigens. Thus, the reduction of the TCR activation threshold and the arming of NKG2D to mediate direct killing in IELs can explain how IELs can kill epithelial cells without recognizing gliadin peptides. Because arming of NKG2D by IL-15 to kill in an antigen non-specific manner can only occur in effector T cells, the questions of what is the initial TCR trigger of IEL activation and whether this trigger is specific to celiac disease are raised. There is presently no direct answer to this question. However, a specific TCR trigger is not required, because IELs are in a *de facto* effector stage because of the high antigenic load present in the intestine. What is not explained by these findings is how IELs can produce IFN-γ and expand. Indeed, NKG2D receptors expressed by celiac IELs are associated with the phosphoinositide 3-kinase binding adapter molecule DAP10, which cannot recruit ζ-associated protein of 70 kDa (ZAP-70) and therefore cannot mediate cytokine secretion and proliferation (16, 18, 19). We have evidence now that NK receptors associated with immunoreceptor tyrosine-based activation motif (ITAM)-bearing adapter molecules, able to mediate these latter functions, can be induced in celiac IELs, and that their ligands are expressed by epithelial cells in active celiac patients (manuscript in preparation). Induction of such receptors may constitute an essential step for chronic activation and proliferation of IELs and hence possibly for their malignant transformation (*Fig.* 4).

Do intraepithelial TCRγδ T cells play a role in celiac disease?

TCRγδ IELs infiltration persists, similarly to IL-15 (21), for a long time before progressively disappearing (93). Furthermore, the stimulation of intestinal organ cultures with gluten induces IL-15 expression on epithelial cells (16), γδ IELs expand rapidly on gluten challenge *in vivo* and *in vitro* (62, 94, 95), and this expansion is IL-15 dependent (95). These observations along with the role played by IL-15 in TCRγδ IEL homeostasis (96) strongly suggest that TCRγδ IEL expansion in celiac disease is driven by IL-15 and not by a specific antigen. This idea is compatible with the finding that there is a preferential non-clonal TCR Vd1 expansion in celiac patients. However, it remains possible that TCRγδ IELs recognize a specific ligand induced on celiac epithelial cells. The function of TCRγδ IELs in celiac disease, independently from the issue of specificity, remains elusive. TCRγδ T cells were shown in mice to express keratinocyte growth factor (97) and to play a role in wound healing (98). However, other functional data show that TCRγδ IEL clones *in vitro* are cytotoxic and display NK-like killing (99). This functional property is further compatible with the expression of NKG2D and CD94 receptors by celiac TCRγδ IELs (18 and unpublished data).

What causes the development of refractory sprue and EATL in celiac patients?

Refractory sprue and EATL are classical and unique complications of celiac disease. There is evidence suggesting that refractory sprue represents a prelymphoma stage that precedes

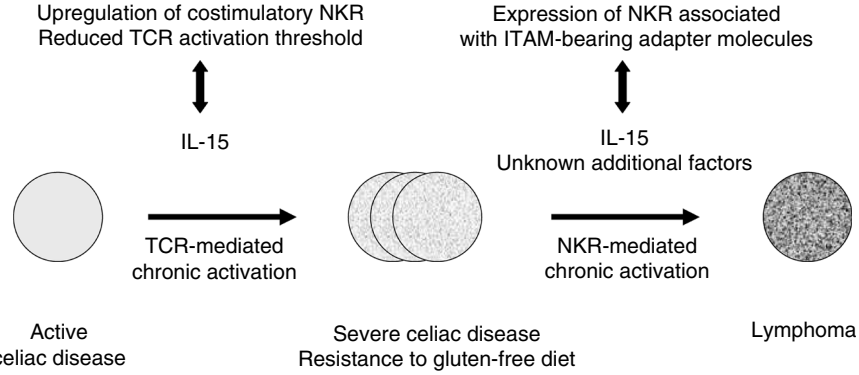

***Fig.* 4. Model accounting for the malignant transformation of intraepithelial lymphocytes (IELs).** Interleukin-15 (IL-15) plays a role in the malignant transformation of IELs by increasing IEL survival and by upregulating the expression of activating natural killer receptors (NKRs) on IELs. IELs acquire costimulatory NKR, which increase T-cell receptor (TCR)-mediated proliferation. Under these conditions of chronic activation, IELs start to express NKR associated with ITAM-bearing adapter molecules, which can induce full activation. This results in TCR-independent, antigen–non-specific NKR-mediated proliferation. Loss of TCR control over IEL proliferation and IL-15 exposure set the stage for the malignant transformation of IELs.

EATL (91, 100–104). Most refractory sprue IELs are highly oligoclonal T cells characterized by the absence of TCR and CD8 surface expression (101). The absence of surface TCR expression is not due to TCR internalization but is secondary to the absence of productively rearranged TCR α/β or γ/δ chains (101 and unpublished data). This phenotype is usually not found in normal IELs, or if present, it is displayed by a very small subset (<5%) of total IELs. Two possible scenarios can account for the absence of TCR surface expression by refractory IELs. In the first scenario, refractory IELs result from the expansion of a rare subset of immature intraepithelial T lymphocytes that never expressed a functional TCR. In the second scenario, refractory IELs are derived from IELs that at some point expressed a functional TCR and lost it after secondary TCR rearrangements upon induction of recombination activating genes under conditions of chronic activation, similarly to what has been described in activated B cells undergoing B-cell receptor editing (reviewed in 105).

The induction of activating NK receptors, which can mediate IEL proliferation independently form TCR activation (manuscript in preparation), may explain how these T cells can continue to expand in the absence of TCR. Furthermore, their expression may constitute a critical step in the malignant transformation of IELs (*Fig.* 4). Finally, IL-15 may play a critical role by promoting the upregulation of activating NK receptors (18, 19, 84, 106) and the survival of IEL (21). Interestingly, hypersecreting IL-15 Tg mice develop CD8 T-cell lymphoma which express NK receptors (107).

Why should a role for gluten in the initiation of an innate immune response be evoked?

The triggering of an innate response by gluten in APCs and epithelial cells can be evoked for the following reasons. First, it could explain why an anti-gluten T-cell response can be mounted. In order to prime naïve T cells, APCs have to be activated by exogenous or endogenous alarm signals to express costimulatory molecules and produce cytokines to drive T-cell polarization (108, 109). There is some evidence that gluten could activate APCs (110, 111). However, these results need to be confirmed, and the molecular basis underlying the activation of APCs by gluten has yet to be determined. Conversely, APC activation and increased intestinal permeability could be induced by viral or bacterial infections, which set the stage for the development of celiac disease.

The potential property of gluten to activate an innate pathway in epithelial cells is supported by the findings that the α-gliadin p31–43 peptide can induce epithelial cell alterations in the absence of $CD4^+$ T-cell activation, whereas peptides inducing $CD4^+$ T-cell activation cannot (15, 16). The innate activation of epithelial cells by gluten is fundamental for celiac disease pathogenesis, because it induces the expression of non-classical MHC class I molecules and IL-15 that are required for the arming of IELs to kill epithelial cells (*Fig.* 3). However, the molecular basis underlying these effects of gluten on epithelial cells have to be identified. Most importantly, the capacity of gluten to activate innate signaling molecules needs to be tested. A key question is whether gluten can trigger an innate signaling pathway in normal individuals or only in celiac patients. In the latter case, genes involved in innate signaling pathways should be linked to celiac disease, similarly to the association of NOD2 with Crohn's disease. Furthermore, it should be possible to identify epithelial stress linked to gluten ingestion in family members of celiac patients in the absence of $CD4^+$ T-cell activation.

Finally, the role of IFN-α in celiac disease remains an opened question. IFN-α is viewed as an innate cytokine able to induce APC activation, IL-15, and IFN-γ production. Surprisingly, IFN-γ expression in celiac mucosa is not associated with IL-12 production (112), suggesting that another cytokine needs to drive IFN-γ production, the most likely candidates being IL-18 and IFN-α. The potential role of IFN-α is supported by the observations that celiac disease has been induced by IFN-α treatment in hepatitis C patients (113–115) and that celiac mucosa of active celiac patients express significant levels of IFN-α. However, IFN-α induction by gliadin in APCs or epithelial cells has not been reported.

Conclusion

There is strong evidence today that both innate and adaptive immunity play a role in the pathogenesis of celiac disease. The emerging concept, which probably applies also to other organ-specific autoimmune diseases, is that T-cell-mediated tissue destruction requires the expression of innate stress signals by tissue cells that arm the $CD8^+$ effector response by upregulating activating NK receptors. The key innate signals expressed by celiac IECs comprise IL-15 and non-classical MHC class I molecules. The challenge will be to determine how gluten induces these and other innate signals and how the $CD4^+$ T-cell response is involved in promoting the effector mechanisms leading to tissue destruction.

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