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Celiac Disease: From Pathogenesis to Novel Therapies

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Celiac disease has become one of the best-understood HLA-linked disorders. Although it shares many immunologic features with inflammatory bowel disease, celiac disease is uniquely characterized by (1) a defined trigger (gluten proteins from wheat and related cereals), (2) the necessary presence of HLA-DQ2 or HLA-DQ8, and (3) the generation of circulating autoantibodies to the enzyme tissue transglutaminase (TG2). TG2 deamidates certain gluten peptides, increasing their affinity to HLA-DQ2 or HLA-DQ8. This generates a more vigorous $CD4^+$ T-helper 1 T-cell activation, which can result in intestinal mucosal inflammation, malabsorption, and numerous secondary symptoms and autoimmune diseases. Moreover, gluten elicits innate immune responses that act in concert with the adaptive immunity. Exclusion of gluten from the diet reverses many disease manifestations but is usually not or less efficient in patients with refractory celiac disease or associated autoimmune diseases. Based on the advanced understanding of the pathogenesis of celiac disease, targeted nondietary therapies have been devised, and some of these are already in phase 1 or 2 clinical trials. Examples are modified flours that have been depleted of immunogenic gluten epitopes, degradation of immunodominant gliadin peptides that resist intestinal proteases by exogenous endopeptidases, decrease of intestinal permeability by blockage of the epithelial ZOT receptor, inhibition of intestinal TG2 activity by transglutaminase inhibitors, inhibition of gluten peptide presentation by HLA-DQ2 antagonists, modulation or inhibition of proinflammatory cytokines, and induction of oral tolerance to gluten. These and other experimental therapies will be discussed critically.

Celiac disease is a common inflammatory disease of the small intestine that is mainly triggered and maintained by the storage proteins (gluten) of wheat, barley, and rye in genetically predisposed individuals. Patients display various degrees of intestinal inflammation, ranging from mere intraepithelial lymphocytosis to severe subepithelial (lamina propria) mononuclear cell infiltration resulting in total villous atrophy coupled with crypt hyperplasia. Accordingly, clinical symptoms and laboratory indices range from completely asymptomatic to global malabsorption.[1–8] Autoantibody screening and biopsy confirmation of celiac disease reveals prevalences in the United States and in most Western and Middle Eastern countries between 1:70 and 1:200.[1–5,9–11] This appears to further increase with age, because a recent study from Finland showed a prevalence of 1:47 in randomly selected subjects older than 52 years of age.[12]

The majority (>80%) of patients with screening-detected celiac disease show no, minor, or non–diarrhea-associated clinical symptoms (clinically silent, oligosymptomatic, or atypical celiac disease, respectively). Oligosymptomatic celiac disease is associated with anemia, osteoporosis, and an often compromised well-being and quality of life,[13] which overlaps with atypical celiac disease that is characterized by extraintestinal symptoms such as arthritis, infertility, hypertransaminasemia, and even liver failure.[1–5,10,11] Furthermore, gluten sensitivity without intestinal lesions but circulating celiac autoantibodies or mere antibodies to gliadin, which lack specificity for classic celiac disease,[14] has been linked to otherwise unexplained neurologic or psychiatric disorders such as cerebellar ataxia, peripheral neuropathy, schizophrenia, or autism.[15–18] Because symptoms in patients may improve on a gluten-free diet, this has led to the suggestion of nutritional gluten sensitivity that does not manifest itself as the classic intestinal lesion but rather as extraintestinal (eg, neurologic disease).[19–21] Its relation to celiac disease is discussed controversially.

Abbreviations used in this paper: CCL25, chemokine ligand 25; CCR, chemokine receptor; EATL, enteropathy-associated T-cell lymphoma; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; PEP, prolyl endopeptidase; TCR, T-cell receptor; TG2, tissue transglutaminase; TGF, transforming growth factor; Th1, T-helper 1; Treg, regulatory T cell.

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Table 1. Non-HLA Loci of Celiac Disease Susceptibility

Loci identified	Type of study used for identification	Origin of the cohort(s)	Candidate genes (function)	Reference
CELIAC 2 5q31-q33	linkage analysis	Italy, Finland, Scandinavia, Europe (meta-analysis)	Unknown	36, 40, 43, 45
CELIAC 3 2q33	Candidate gene approach	France, The Netherlands, Sweden, Norway	CTLA4 (T cell response)	38, 41, 48
CELIAC 4 19p13.1	linkage analysis	Netherland	Myosin IXB (Rho family guanosine triphosphatase)	44
CELIAC 5 15q11-q13	linkage analysis (microsatellite)	Finland	Unknown	49
CELIAC 6 4q27	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Italy, United States, Scandinavia	KIAA1109 TENR (ADAD1) IL2 IL21	31, 33, 35, 39, 47
CELIAC 7 1q31	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Italy, United States	RGS1 (B-cell activation)	31, 33, 39, 47
CELIAC 8 2q11-q12	GWAS (SNPs)	United Kingdom, Netherland, Ireland	IL18RAP IL18R1	31,33, 42
CELIAC 9 3p21	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Spain	CCR1 (chemokines) CCR2 CCRL2 CCR3 CCR5 XCR1	31, 33, 37
CELIAC 10 3q25-q26	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Italy, United States	IL12A	31, 33, 39, 47
CELIAC 11 3q28	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Italy, United States	LPP (zinc binding protein)	31, 33, 39,47
CELIAC 12 6q25.3	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Italy	TAGAP (T cell activation)	31, 33, 47
CELIAC 13 12q24	GWAS (SNPs)	United Kingdom, Netherland, Ireland, Italy, United States	SH2B3 (TLR intracellular adaptor, T-cell activation)	31, 33, 39, 47

GWAS, genome-wide association study; SNP, single nucleotide polymorphism.

Classic celiac disease is frequently found in conjunction with (other) autoimmune diseases, such as type 1 diabetes mellitus, autoimmune thyroiditis, autoimmune hepatitis, dermatitis herpetiformis, and autoimmune alopecia.[22] In addition, patients with long-standing undetected and untreated symptomatic celiac disease are at an increased risk for developing enteropathy-associated T-cell lymphoma, small bowel adenocarcinoma, and other cancers of the gastrointestinal tract.[23–26] If and to what extent patients with silent or oligosymptomatic screening-detected celiac disease may develop overt celiac disease, secondary autoimmune diseases, or even malignancy when continuing on a gluten-containing diet remains to be shown.

The only currently available treatment of celiac disease is a lifelong strict gluten-free diet, which is difficult to maintain and can lead to social isolation because modern diets are heavily based on products that contain gluten.

Pathogenesis of Celiac Disease

Virtually all patients with celiac disease share the heterodimeric HLA class II genes HLA-DQ2 or HLA-DQ8 as common genetic background. These class II molecules are expressed on antigen-presenting cells, mainly macrophages, dendritic cells, and B cells. Gluten peptides are presented by these celiac disease–associated HLA class II molecules. This can lead to activation of gluten-specific $CD4^+$ T-helper 1 (Th1) cells in the lamina propria that are central effector cells of the intestinal inflammation resulting in crypt hyperplasia and villus atrophy.[6,27] However, HLA-DQ2 or HLA-DQ8 are expressed in 30%–35% of the populations where celiac disease is prevalent, with only ~2%–5% of gene carriers developing celiac disease. This implicates other genetic as well as environmental factors as contributors to the manifestation of celiac disease.[28,29] Recent genetic studies in large numbers of patients with celiac disease, relatives, and matched controls revealed additional risk factors, most of which are related to T-cell regulation and inflammation.[30–33,35–49] However, the overall genetic contribution of these polymorphisms combined was estimated at only 3%–4% as compared with 30%–35% for HLA-DQ2 or HLA-DQ8.[33,34] The 13 susceptibility loci that have been identified to date are summarized in Table 1.[31,33,35–49] Furthermore, early exposure of infants to dietary gluten,[50] early infection with enteropathic viruses, or a change of the bacterial flora[51–56] were shown to favor the evolution of clinically manifest celiac disease in childhood. These observations indicate that celiac disease results from dys-

regulation of a usually suppressed T-cell response to gluten in a subset of carriers of HLA-DQ2 or HLA-DQ8.

Almost all patients with celiac disease develop immunoglobulin (Ig) A autoantibodies to the enzyme tissue transglutaminase (transglutaminase 2 [TG2]),[57,58] which is expressed by many cell types and associates with the extracellular matrix (endomysium or reticulin fibers). TG2 targets certain glutamine residues in some extracellular and intracellular proteins, usually tethering them to a lysine residue of a second protein that results in cross-linking of both proteins. Alternatively, TG2 merely deamidates these glutamines to negatively charged glutamic acid residues.[59–61] Due to their high content in glutamine and neighboring proline and hydrophobic amino acid residues, gluten proteins, especially the alcohol-soluble fraction (ie, gliadins of wheat, secalins of barley, and hordeins of rye) but also the glutenins, are preferred substrates for TG2.[57,62] When deamidated, most of the resultant negatively charged gluten peptides bind more strongly to HLA-DQ2 (or HLA-DQ8), which leads to a more rigorous gluten-specific $CD4^+$ Th1 T-cell activation. A large number (>50) of distinct (deamidated) gluten peptides that can trigger such T-cell responses have been identified or deduced from consensus sequences for TG2.[63–72] These gluten peptides are usually fairly resistant to digestion by gastrointestinal proteases, which increases their survival and availability in the small intestine.[67,69] A 33mer peptide from α2-gliadin contains 6 partly overlapping HLA-DQ2–binding amino acid sequences that can be deamidated by TG2.[68] This peptide is considered a celiac "superantigen" and used as a model peptide in preclinical studies.

How the immunogenic gluten peptides reach the lamina propria from the intestinal lumen remains controversial. There is evidence that they can traverse via a paracellular pathway through defective tight junctions,[73] but other studies showed that much of the transport occurs via epithelial transcytosis, especially in the inflamed mucosa of patients with celiac disease.[74–77] How far an association of gluten peptides with luminal anti-gluten IgA and retrotranscytosis from the apical to the basal pole of the epithelium may contribute to transcytosis in vivo remains to be shown.[77] A third but yet unproven possibility is the sampling of gluten peptides by lamina propria dendritic cells. It was shown in mice but not in humans that these cells can project protrusions between intestinal epithelial cells reaching the intestinal lumen.[78] Similarly, sampling of gluten peptides by dendritic cells could occur preferentially via specialized microfold cells that are part of the follicle-associated epithelium of the mucosa-associated lymphoid tissue.[79]

Innate Immunity to Gluten

While the adaptive immune response to gluten is well established, proteins from wheat, rye, or barley (apparently in contrast to "nontoxic" cereal proteins derived from, for example, corn or rice) can elicit an innate immune response in professional antigen-presenting cells (monocytes, macrophages, and dendritic cells) that activates predominantly intraepithelial lymphocytes (IELs) but also intestinal epithelial cells.[80–84] This innate immune response is an immediate reaction and is usually directed against relatively uniform microbial antigens but also against yet ill-defined constituents of cereals.[85,86] In celiac disease, the innate immune response appears to favor the development of adaptive immunity to gluten in patients that carry HLA-DQ2 or HLA-DQ8.[81] α2-gliadin peptide p31-43, which is distinct from peptides that elicit adaptive immunity, was shown to trigger innate immunity in intestinal epithelia and intestinal organ cultures.[81,87] Other peptides reportedly stimulated rodent monocyte or macrophage cytokine release.[80,82,83] However, these peptides have not been generally confirmed and none of the studies identified a receptor on a responsive cellular subset. Two recent studies that rigorously ruled out contamination by lipopolysaccharide implicated MyD88, the major downstream signal transducer of Toll-like receptor 4 on monocytes, macrophages, and dendritic cells, and Toll-like receptor 4 itself as primary receptor for innate responses to cereal proteins.[84,88]

The Role of IELs

Progress has been made in our understanding how IELs are activated by luminal cereal proteins. The perforin/granzyme and/or Fas/FasL pathways are central to the observed cytotoxicity and apoptosis-inducing activity of IELs on the intestinal epithelium in celiac disease.[89–91] Innate immune activation of IELs by gluten induces expression of the nonclassic class I molecule MICA on the intestinal epithelium, which serves as ligand for the heterodimeric NKG2D receptor on natural killer, $\gamma\delta$ T cells and on subsets of $CD4^+$ and $CD8^+$ T cells.[92] Epithelial MICA and up-regulated epithelial production of interleukin (IL)-15 leads to activation of NKG2D on IELs.[93] NKG2D also links innate and adaptive immunity, because it both triggers antigen-specific lymphocyte–mediated cytotoxicity and induces a direct cytolytic function independent of T-cell receptor (TCR) specificity in effector CD8 T cells.[94] Similarly, the NKG2C receptor that is activated by its epithelial ligand HLA-E is implicated in the pathogenesis of celiac disease, stimulating IEL proliferation and cytokine secretion in patients with celiac disease.[95–97] IELs can also have an immunoregulatory capacity through the secretion of transforming growth factor (TGF)-β1, as reported for a subset of $CD8^+TCR\alpha\beta^+$ IELs that express the inhibitory NK receptor NKG2A. Interestingly, this subset of regulatory cells was reduced in duodenal biopsy specimens from patients with active celiac disease as compared with controls or patients on a gluten-free diet.[98]

The central role of IL-15 in the activation of innate and adaptive immunity in celiac disease has been confirmed

Figure 1. Pathogenesis of celiac disease. Gluten peptides that are highly resistant to intestinal proteases reach the lamina propria, via either epithelial transcytosis or an increased epithelial tight junctional permeability. Cross-linking and particularly deamidation of gluten peptides by TG2 creates potent immunostimulatory epitopes that are presented via HLA-DQ2 or HLA-DQ8 on antigen-presenting cells. Subsequently, $CD4^+$ T cells are activated, secreting mainly Th1 cytokines such as IFN-γ, which induces the release and activation of MMPs by myofibroblasts, finally resulting in mucosal remodeling and villus atrophy. Additionally, Th2 cytokines are produced driving the production of (auto-)antibodies to gluten and TG2. Other cytokines such as IL-18, IFN-α, or IL-21 seem to play a role in polarizing and maintaining the Th1 response. Furthermore, IL-15 links the adaptive immune system to innate immune responses (see Figure 2). The scheme is simplified. It does not show that T cells circulate to mesenteric lymph nodes where they encounter and are primed by antigen-presenting cells (mainly dendritic cells) and from where they home back to the lamina propria, a process that is driven by the lymphocyte homing receptors CCR9 and integrin $\alpha4\beta7$.

by several investigators,[90,99–102] coupled with an increased expression of IL-15 receptor and a lower threshold for activation on IELs.[100] Both intestinal epithelia and dendritic cells/macrophages are major sources of IL-15.[90,103] Apart from being a potent growth factor for IELs, IL-15 blocks Smad3-dependent transcription via the activation of c-Jun-N-terminal kinase and phosphorylation of c-jun and thus counteracts the immunosuppressive TGF-β pathway.[99] Recently, IL-21, which is produced by $CD4^+$ Th1 T cells, has emerged as an additional driving force of innate immunity that often acts in concert with IL-15.[104] Figures 1 and 2 summarize key concepts of the pathogenesis of celiac disease.

Regulatory T Cells

$CD4^+$ regulatory T cells (Tregs) can down-regulate destructive T-cell responses, either in autoimmunity or infection. Although their role in murine models of autoim-

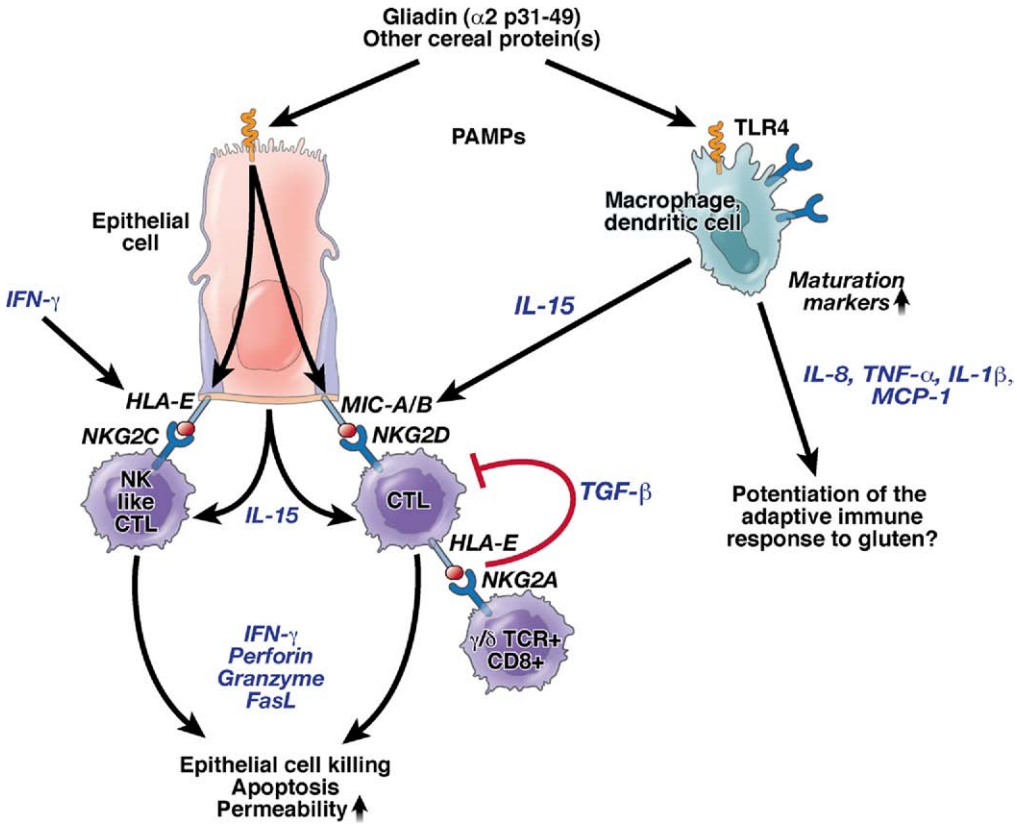

Figure 2. Innate immune responses in celiac disease. Upon stimulation with gliadin peptide p31-49 (and other peptides), epithelial cells, macrophages, and dendritic cells secrete IL-15, which in turn up-regulates both the NKG2D receptor on IELs and its epithelial ligand MICA. The thus stimulated cytotoxic lymphocytes induce increased epithelial apoptosis and permeability. Furthermore, the NKG2C receptor on a subset of natural killer–like IELs is stimulated by its epithelial ligand HLA-E on epithelial cells, resulting in their proliferation and cytotoxicity, whereas stimulation of $\gamma\delta^+$ $CD8^+$ IELs bearing the NKG2A receptor via HLA-E induces TGF-β secretion and therefore a regulatory phenotype. Gliadin (cereal) peptides can also directly elicit innate immune responses in macrophages and dendritic cells via pattern recognition receptors such as Toll-like receptor 4 or other MyD88-dependent pathways. This drives maturation of these cells and secretion of inflammatory cytokines such as IL-1β, IL-8, tumor necrosis factor α, and MCP-1, which can potentiate the adaptive immune response to gluten. APC, antigen presenting cell; pDC, plasmacytoid dendritic cell.

munity and inflammatory bowel diseases is well established,[105] their relevance as suppressors of human T cell–mediated disease is just emerging. $CD4^+CD25^+Foxp3^+$ Tregs have been shown in peripheral blood mononuclear cells and in intestinal biopsy specimens of patients with celiac disease, but functional studies are lacking.[106,107] In mouse models, these cells are generated in the periphery (predominantly in mesenteric lymph nodes) and to a lesser degree in the intestine (Peyer's patches and the lamina propria) from naive $CD4^+$ T cells in the presence of TGF-β1 and retinoic acid.[108] This maturation occurs in the presence of (retinoic acid–producing) dendritic cells that present the target antigen, followed by homing of the Tregs mainly to the gut where they down-regulate immune responses. The process is dependent on the chemokine receptor (CCR) 7 on dendritic cells (for homing to mesenteric lymph nodes and Peyer's patches) and on CCR9 and the integrin $\alpha4\beta7$ on the T cells for their homing to the intestinal lamina propria via adherence to the $\alpha4\beta7$ ligand MadCAM on high endothelial venules.[109–111] Interestingly, when retinoic acid is substituted by IL-6, naive T cells are converted to destructive Th17 cells.[108,112]

$CD8^+$ Tregs have only recently reemerged as important suppressors of intestinal immune responses, largely due to a better understanding of underlying mechanisms.[113,114] Like the $CD8^+$ cytotoxic T cells that are implicated in mucosal destruction, both in inflammatory bowel disease and celiac disease, the $CD8^+$ Tregs mainly reside in the epithelial compartment of the intestine as IELs. In mouse models of inflammatory bowel disease, the cytotoxic $CD8^+$ IELs appear to initiate and maintain the destructive, $CD4^+$ T cell–mediated immune response within the lamina propria, leading to a breach of the mucosal barrier, entry of luminal antigens, and massive stimulation of a $CD4^+$ Th1 T-cell response.[115–117] These aggressive IELs express the TCR $\alpha\beta$ or $\gamma\delta$ heterodimer in conjunction with the CD8$\alpha\beta$ heterodimer. In contrast, $CD8^+$ Tregs express FoxP3 and the CD8$\alpha\alpha$ homodimer. Generation of these Tregs apparently occurs during thymic selection in the presence of cognate antigen when CD8$\alpha\beta$ are deselected.

Although some mechanisms need confirmation in the human system and particularly in celiac disease, the improved understanding of mucosal immunology opens

the possibility for a causal treatment aimed at inducing tolerance to ingested gluten (see the following text).

Cytokines and Matrix Remodeling in Celiac Disease

HLA class II–restricted gliadin-specific T-cell clones express interferon (IFN)-γ. An IFN-γ blocking antibody can prevent histologic damage to healthy mucosa in an intestinal organ culture system exposed to supernatants of gliadin-specific T-cell clones from patients with celiac disease,[118] whereas IL-10 from Tregs suppresses Th1 cells and likely acts as a mildly counterregulatory cytokine.[119] Cytokines are important driving forces of tissue remodeling that result in the villus atrophy and crypt hyperplasia that are characteristic of celiac disease. In human fetal intestinal explant cultures, IFN-γ activates macrophages that in turn secrete tumor necrosis factor α and proteolytic matrix metalloproteinases (MMPs), such as MMP-12 and MMP-13. In intestinal myofibroblasts, both TNF-α and IFN-γ stimulate the expression of proteolytic MMP-1 and MMP-3. This composite MMP release and activation induces extracellular matrix proteolysis, a precondition for the architectural remodeling observed in inflammatory bowel disease and celiac disease.[120–122] As in Crohn's disease, these MMPs may represent a therapeutic target.[123]

Refractory Celiac Disease and Intestinal T-Cell Lymphoma

Refractory celiac disease can develop in 5%–10% of adults with long-standing (often undetected) celiac disease. Patients with refractory celiac disease do not respond to or experience a relapse while on a strictly gluten-free diet. The diagnosis of refractory celiac disease can only be made when (inadvertent) gluten ingestion or other diseases that can cause diarrhea and villus atrophy have been ruled out. Thus, 82%–90% of patients with "refractory" celiac disease referred to 2 large tertiary care centers had proven gluten ingestion or an incorrect diagnosis.[124,125]

Refractory celiac disease is now classified as refractory celiac disease type 1 and 2.[126–129] Refractory celiac disease type 1 is responsive to corticosteroids and other immunosuppressants and only rarely evolves into enteropathy-associated T-cell lymphoma (EATL). In contrast, refractory celiac disease type 2 can be considered a premalignant condition, and roughly 50% of patients with refractory celiac disease type 2 develop EATL within 5 years of diagnosis.[126–129] Patients with refractory celiac disease type 2 and EATL frequently have lost autoantibodies to TG2 and display clonal growth of (intraepithelial) natural killer and cytotoxic T cells that primarily belong to the innate immune system.[85,130] Normally, 70% of IELs express the suppressor/cytotoxic phenotype CD8 and only 5%–10% express the helper CD4 phenotype. In refractory celiac disease type 2 and EATL, immunohistochemistry reveals infiltration of the intestinal epithelium by small lymphocytes that lack expression of CD8, CD4, and TCRs while they retain intracytoplasmic but not surface staining for the general T-cell marker CD3. Furthermore, polymerase chain reaction for TCR-γ gene rearrangements can be performed from biopsy specimens that shows monoclonality, and flow cytometry of duodenal T cells can diagnose refractory celiac disease type 2 and EATL when the fraction of aberrant T cells exceeds 20%.[131] In analogy to other lymphomas, treatment of EATL (and refractory celiac disease type 2) is based on cytotoxic agents such as cladribine, but results for EATL are disappointing.[126] However, autologous and especially allogeneic bone marrow transplantation offer hope.[132]

Recent data indicate a relative risk of ~3 for patients with (untreated) celiac disease to develop EATL,[25,133] which is much lower than in previous studies. This is likely due to the much higher (5- to 13-fold) prevalence of silent or atypical celiac disease on which today's studies are based when compared with earlier studies that used classic celiac disease.[134] When patients are on a gluten-free diet for 5 years or more, the risk of developing lymphoma (and gastrointestinal cancers) appears to approach that of the normal population.[25,133]

Malignant clones of original IELs may still depend on innate immune triggers, and their downstream signal transduction, such as IL-15 and IL-21 (see previous text), is implicated in refractory celiac disease type 2 and EATL.

Preclinical Models of Celiac Disease

These models have been highly useful to explore the pathogenesis of celiac disease and assess the efficacy of nondietary therapies. They will be discussed in brief, because they have recently been reviewed in detail.[135]

In Vitro Models

Culture of intestinal biopsy specimens from patients with celiac disease has been used as a plausible approximation to the in vivo disease process. Exposure of the biopsy specimens to a peptic-tryptic digest of gluten or gliadin replicates some of the pathology that is found in vivo, such as intestinal epithelial apoptosis and intraepithelial and lamina propria T-cell activation, even villus atrophy, and secretion of autoantibodies to endomysium (TG2) into the culture medium.[136–138] However, interpretation of the results is limited by the harsh conditions imposed by ex vivo organ culture, leading to hypoxic damage, especially of noninflammatory cells, usually within 1–2 days. Nonetheless, it may yield important information that can only be obtained in a multicellular context, such as the proinflammatory role of IFN-γ[118] and IL-15[81,101] or the anti-inflammatory activity of IL-10.[91]

Gluten-Reactive T-Cell Clones

Intestinal T cells from patients with active celiac disease can mount a strong adaptive Th1 (IFN-γ dominated) response to certain gluten epitopes, especially those generated by TG2-mediated deamidation. Ex vivo expanded T-cell lines and especially T-cell clones against an increasing spectrum of these epitopes have become highly useful in (1) confirming the uniform HLA-DQ2 or HLA-DQ8 dependency of adaptive immunity in celiac disease; (2) demonstrating the large number of distinct T-cell epitopes within wheat, barley, and rye, including glutenins as well as gliadins; (3) allowing a comparison of the potency of the epitopes to trigger T-cell activation; and (4) elucidating epitope spreading from infancy to adulthood.[63–72] Furthermore, they permit the testing of novel therapies that are aimed at inactivation of antigenic T-cell epitopes in cereals or at inhibition of the DQ2 or DQ8 molecules on the surface of antigen-presenting cells (see the following text).

Animal Models of Celiac Disease

The Irish setter can develop mucosal atrophy in response to wheat ingestion,[139] but the pathogenesis is unlike celiac disease; because disease does not develop when the first gluten exposure occurs after an age of 8–9 months,[140] villous atrophy is not linked to major histocompatibility complex class genes and no serum antibodies to gluten can be detected.[141]

Because all patients with celiac disease bear HLA-DQ2 or HLA-DQ8, HLA-DQ2 or HLA-DQ8 transgenic mice should render suitable models that replicate the pathogenesis of celiac disease. Several transgenic mice have been developed that express human CD4 and DQ8 in the absence of their murine counterparts that would interfere with human immunology.[142,143] After being immunized with gliadin, the T cells of these mice showed in vitro responses to gluten in a HLA-DQ8– and CD4-restricted manner, whereas T cells from HLA-DQ6 $CD4^+$ control mice did not develop a gliadin-specific immune response.[142,144,145] However, apart from high levels of anti-gliadin IgG antibodies, the mice did not show any celiac pathology.[142,144,145] The cytokine profile in these mice resembled that of a regulatory phenotype, characterized by $CD4^+CD25^+$ T cells and production of IL-10 and TGF-β1,[142] likely leading to tolerance to gliadin, whereas celiac disease is driven by a Th1 response dominated by IFN-γ. Furthermore, mice did not have circulating anti-TG2 or IgA anti-gliadin antibodies. The same group crossed human HLA-DQ8 into nonobese diabetic mice.[146] Sensitization of these mice with gluten did not cause intestinal pathology, but 15 out of 90 animals developed blistering skin lesions resembling those of dermatitis herpetiformis, a disorder that occurs in up to 10% of patients with celiac disease. However, affected mice did not develop IgA antibodies to gliadin or antibodies to TG2.

Given the fact that >90% of all patients with celiac disease possess HLA-DQ2 whereas only 5%–10% bear HLA-DQ8,[147] in vivo studies in mice transgenic for human CD4 and HLA-DQ2 are attractive. However, similar to the results in HLA-DQ8 transgenic mice, and even after coimmunization with pertussis toxin, only 2 out of 14 gluten-fed HLA-DR3-DQ2 transgenic mice developed IgA autoantibodies to TG2 and only 2 animals developed an increase in IELs. Furthermore, backcrossing the mice to a nonobese diabetic background or generating mice transgenic for a gliadin-specific TCR did not lead to intestinal or dermal pathology.[148,149]

Spontaneously occurring gluten sensitivity was detected in 3% of a rhesus macaque strain. Upon oral gluten ingestion, the affected monkeys developed small intestinal pathology reminiscent of celiac disease, combined with malabsorption and weight loss. Affected monkeys recovered after reinstitution of a gluten-free diet.[150,151] Gluten-sensitive animals had circulating IgA and IgG antibodies to gliadin, and 3 of 15 displayed mildly elevated IgG anti–TG2 levels. A problem is the rare spontaneous occurrence of the complete celiac disease phenotype (0.6%) and the animal species (primates), which currently precludes large-scale exploration of novel nondietary therapies in this model.

Recently, a celiac disease mouse model was established by transfer of presensitized effector/memory T cells ($CD4^+CD45RBlowCD25^-$) from gliadin-immunized wild-type mice to T cell– and B cell–deficient ($Rag1^{-/-}$) or T cell–deficient (nude) mice.[152] Recipient mice gained less weight and experienced severe duodenitis upon challenge with oral gluten when compared with recipients on a gluten-free diet or compared with recipients of control (ovalbumin)-presensitized T cells. This was accompanied by mucosal histologic features characteristic of celiac disease (villous atrophy and crypt hyperplasia) and a Th1/Th17 T-cell polarization in the duodenum and the periphery. Reintroduction of a gluten-free diet led to weight gain, improvement of histologic duodenitis, and a decrease in duodenal proinflammatory transcripts. Moreover, B cell–competent nude recipients of gliadin-presensitized effector/memory T cells produced high levels of serum anti-gliadin IgA and IgG1/IgG2c only when challenged with oral gluten. Although further refinement towards an HLA-DQ2 or HLA-DQ8 background is desirable, this model should be useful for the study of the breach of oral tolerance in celiac disease and for preclinical testing of novel nondietary therapies for celiac disease.

Diagnostic Methods to Assess Therapy Response

Duodenal histology showing intraepithelial lymphocytosis, crypt hyperplasia, and various degrees of villous atrophy, coupled with clinical signs and laboratory parameters of malabsorption, can still be considered the

gold standard to assess the severity of celiac disease. Histology should be performed on ≥6 biopsy specimens from all quadrants of the proximal small intestine, and specimens have to be correctly oriented.[1–7] Rigorous testing of novel therapies for celiac disease still requires a pretreatment and posttreatment assessment using these parameters in large numbers of patients in remission who receive either gluten alone or gluten with the novel therapy. However, changes of these parameters may occur within a few days in some patients and within weeks or months in others. Furthermore, due to focal disease, there may be sampling error even when several biopsy specimens are taken. Staining for and semiquantification of immune activation markers in biopsy specimens, such as IFN-γ, tumor necrosis factor α, TGF-β, IL-2, IL-6, IL-10, and IL-15, both at the RNA and protein level,[118,153–155] may be useful but has not been validated in clinical studies. The same applies to quantitative polymerase chain reaction for genes encoding these cytokine markers and certain MMPs.[122,154,156,157] Although autoantibodies to TG2 or antibodies to deamidated gliadin peptides are excellent tools to detect patients with untreated celiac disease or diagnosed patients with frequent gluten exposure, and antibody titers show some correlation with histologic or clinical severity,[158–162] they are not sensitive enough for early detection of (minor) gluten exposure. In addition, they lack sensitivity to detect therapeutic effects due to their long half-life (at least 30 days).[163,164] Immunohistologic detection of IgA anti-TG2 deposits in intestinal biopsy specimens precedes the appearance of serum autoantibodies[165] (see also Figure 1), but they may persist despite the lack of other histologic abnormalities, making the test unsuitable for therapeutic studies. Therefore, alternative and preferably noninvasive methods are urgently needed.

Fecal Fat and Sugar Absorption Tests

A 3-day fecal fat collection is an accurate quantitative test for malabsorption, but most patients with celiac disease do not have steatorrhea. Equally, the sensitivity and specificity of oral sugar tests, such as D-xylose absorption, is low, even in many patients with classic celiac disease.[166] Both parameters were measured in patients in remission before and during a 21-day moderate gluten challenge (5–10 g/day).[167] Although tests were pathological in most patients after 15 days of gluten challenge, roughly 50% had already pathological baseline results. The low sensitivity and specificity of these tests were confirmed in the first clinical trial with oral prolyl endopeptidase to digest immunogenic gluten epitopes.[167]

The absorption of usually nonabsorbable versus absorbable sugars has been used to reflect small intestinal epithelial (tight junctional) leakiness, as occurs in Crohn's disease and celiac disease. An early study in 17 patients with celiac disease and 12 controls showed an excellent predictive value of the absorbed lactulose/mannitol uptake for predicting villus atrophy,[168] and the test was recommended as a good screening tool for celiac disease in 111 first-degree relatives of patients with celiac disease.[169] In a pilot study that assessed the paracellular permeability inhibitor AT-1001, 70% of the 14 patients with celiac disease in remission but none of the 7 controls who were exposed to 3 g of gluten showed an increased lactulose/mannitol ratio.[170] However, the sensitivity of the test was questioned in a larger dose-escalation study of AT-1001.[171]

Clinical Scores

Clinical scores are an important adjunct to studies evaluating novel therapeutics. To date only symptom scores that were derived from general health queries for other gastrointestinal diseases were evaluated in celiac disease, lacking important disease-specific characteristics.[172,173] A celiac disease–specific celiac symptom index was recently validated in 154 patients based on 16 items that correlated highly with general health and adherence to the gluten-free diet.[174] The celiac symptom index will serve as an important adjunct tool for future clinical studies.

Follow-up of T-Cell Activation With HLA-DQ2 (DQ8) Tetramers and IFN-γ ELISpot

Based on the identification of several gluten epitopes recognized by T cells from patients with celiac disease,[63,64,175–179] gliadin peptide-DQ2 tetramers that activate human T-cell clones were developed.[180] When used in flow cytometry, these tetramers detected and quantified gluten-specific CD4 T cells in the peripheral blood of patients with celiac disease after a short-term bread challenge,[181] making this technology an attractive tool to detect early gluten responses (eg, in clinical trials). However, tetramers cannot assess T-cell function, the appearance of tetramer-positive T cells in peripheral blood is transient, occurring only within 3–6 days after short-term gluten challenge, and tetramer staining of activated T cells is quickly diminished due to antigen-induced down-regulation of the TCR.[63] In contrast, the IFN-γ ELISpot permits functional characterization but is equally limited by the transient nature of the peripheral T-cell response.[182–184]

Search for Better Serum Markers via Proteomics

Proteomic projects to detect novel serologic markers of celiac disease activity are attractive, but no data for celiac disease have been published to date. They are based on the serum proteome of patients with celiac disease in remission who are challenged with gluten. The serum proteome of the patients before and after challenge can be interrogated using several approaches that are based on depletion of abundant proteins, protein fractionation, mass spectrometry, and bioinformatics.[185–187]

Novel Therapies for Celiac Disease

An effective therapy for patients with celiac disease is adherence to a strict gluten-free diet, which often restricts social activities, limits nutritional variety, is costly, and is difficult to maintain in many countries. Furthermore, a sizable proportion of patients with high-level gluten sensitivity, possibly including patients with proven refractory celiac disease type 1, are affected by trace amounts of gluten in foods that are declared gluten free. Therefore, alternative or adjunctive treatments are desired and necessary.[8,9,188] Such treatments should be of low risk and reasonable cost and lend moderate to high efficacy for the majority of patients. Their major realistic aim would be the "neutralization" of low amounts of gluten (eg, up to 3 g/day as compared with the 13–15 g/day in a normal Western diet) to protect patients from minor unintentional or unavoidable gluten ingestion. In patients with refractory celiac disease, effective therapies that are more costly and incur a higher risk are acceptable, because these patients have few alternatives. The same would apply to a curative (immunologic) approach, even in patients with classic celiac disease.

The following text discusses therapeutic strategies that have been tested in in vitro or in vivo models of celiac disease and approaches that may be promising in the near future. Therapies can be subdivided as to their intraluminal, epithelial, or subepithelial action (Figure 3 and Table 2).

Intraluminal Therapies

Intraluminal therapies are focused either on reducing gluten immunogenicity or on sequestering gluten to prevent its uptake across the intestinal epithelium.

Wheat variants and genetic modification. Wheat strains with lower immunogenicity (ie, a decreased number of immunogenic T-cell epitopes) can either be selected from already existing varieties or created by genetic modification. Ideally, this should lead to preservation of the desired baking properties. The hexaploid *Triticum aestivum* is the most widely used wheat variety in the food industry. It was generated by hybridization between tetraploid *Triticum turgidum* (genes AABB, "pasta wheat") and the diploid *Triticum tauschii* (genes DD). The tetraploid *Triticum turgidum* likely originated from the wild-growing diploid *Triticum monococcum* (AA genome) and *Triticum speltoides* (BB genome).[189] Using duodenal biopsy specimens from patients with celiac disease, a peptic-tryptic digest of tetraploid wheat gluten showed decreased toxicity when compared with a digest of hexaploid wheat.[190] Similarly, 2 wheat varieties, one poor in α and β gliadins and the other in α, β, γ, and ω gliadins, revealed decreased toxicity on duodenal biopsy specimens.[191]

The availability of gluten-specific T-cell clones from duodenal biopsy specimens of patients with celiac disease and the identification of key immunogenic T-cell epitopes, including specific antibodies directed to some of these epitopes,[64,67,71,179,192] provided reproducible tools for the characterization of less toxic wheat species. Thus, 16 wheat varieties (diploid, tetraploid, and hexaploid) displayed highly variant abilities to trigger the activation of T-cell clones that was independent of the ploidy of the genomes but correlated with the presence of specific epitopes derived from α-gliadin, γ-gliadin, and low-molecular-weight glutenin.[193] Interestingly, the gluten digest from *T tauschii* (DD genome), which contains sequences of the 33mer T-cell "superantigen" from α-gliadin,[68] that is encoded by the D genome elicited the strongest T-cell responses, whereas T-cell responses to gluten derived from the AA and BB genome species that lack these sequences were dampened.[194] These findings were confirmed by an in silico approach that analyzed 230 α-gliadin sequences derived from ancestral haplotypes for the presence of T-cell stimulatory epitopes that bind to HLA-DQ2/8, with all major immunogenic peptides present in the DD genotype except for α9 sequences in the AA genotype.[195] Similarly, gliadin derived from *T monococcum* was unable to induce cellular damage on intestinal T-cell lines[196] or IFN-γ production and histologic damage in duodenal biopsy specimens from patients with celiac disease.[197]

The effect of genetic deletion of certain gliadin genes has been analyzed in deletion lines of *T aestivum* (cultivar Chinese Spring), which lack one locus containing gluten genes, by using in silico analysis based on the known DNA sequences and by Western blotting with epitope-specific antibodies.[198] Complete deletion of the α-gliadin locus on chromosome 6 led to a decrease in total T-cell stimulatory epitopes but also impaired the baking properties, whereas deletion of γ-gliadins, ω-gliadins, and low-molecular-weight glutenins on chromosome 1 lowered the immunostimulatory capacity without compromising baking properties. The investigators concluded that deleted gliadins need to be replaced by nonimmunogenic gliadin variants or avenins (the largely nonimmune stimulatory prolamins from oats), because an altered ratio of gliadins to glutenins changes dough elasticity.

Using a similar approach, wheat varieties characterized by a reduced function in the enzymes involved in gliadin and low-molecular-weight glutenin synthesis can be identified. Such varieties can also be generated by mutagenesis, using radiation, azide treatment, or site-directed mutagenesis, but significant effort is needed for the screening of numerous recombinants. The TILLING (targeting induced local lesions in genomes) approach allows screening of a large number of mutagenesis-induced recombinants based on the known sequence and the use of endonucleases to identify the presence of mutations,[199–203] whereas ecoTILLING detects the presence of natural variants. Here, the 5-methylcytosine deglycosylases of wheat represent an attractive target. These enzymes have to demethylate the promoters of all gliadin and low-molecular-weight glutenin genes before their

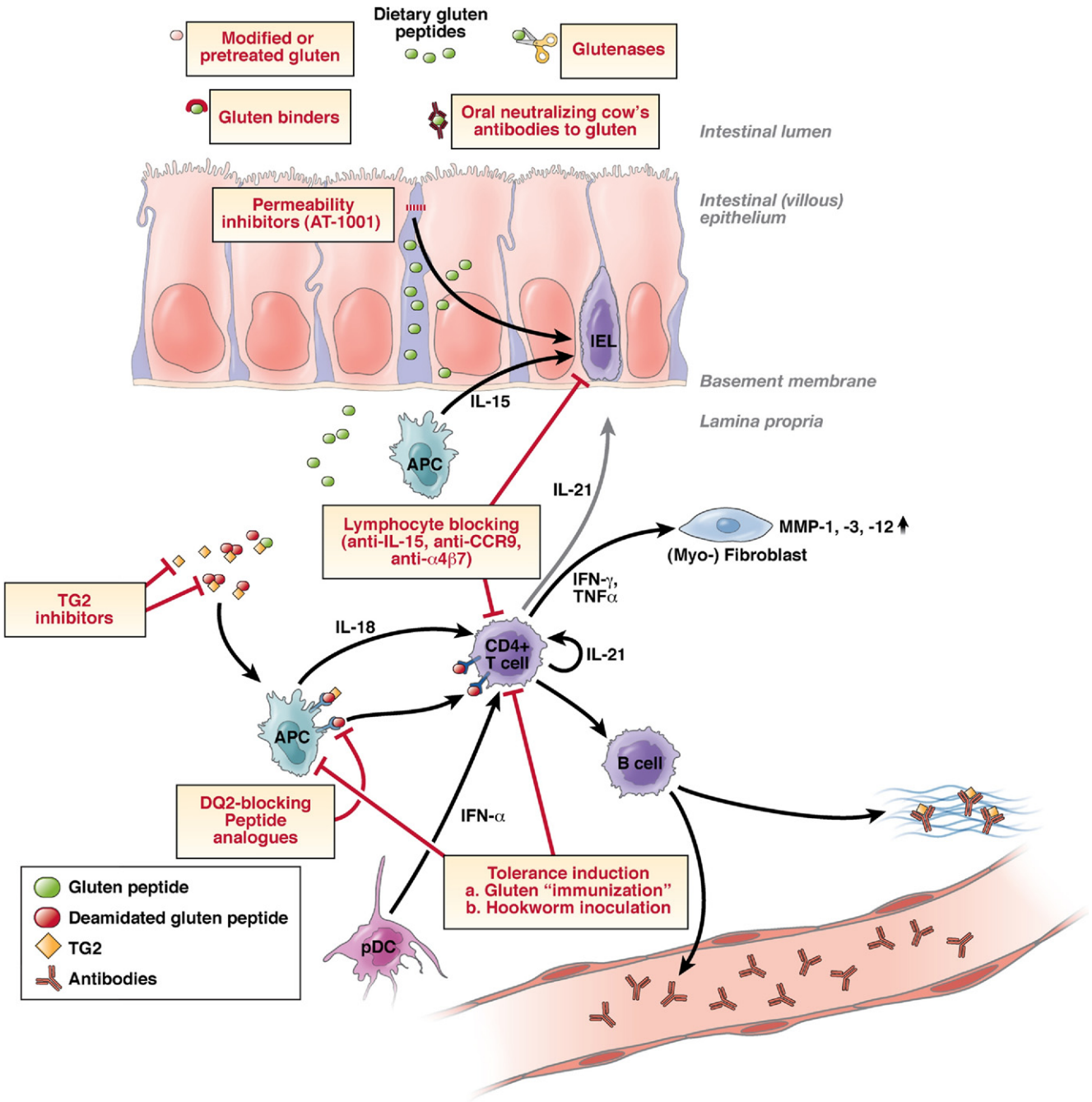

Figure 3. Novel therapeutic approaches. Use of ancestral and/or modified wheat strains with lower immunogenicity. Intraluminal therapies that either bind or degrade ingested gluten peptides in the intestine (glutenases, gluten binders, neutralizing antibodies). Blocking the ZOT receptor with the octapeptide AT-1001 to decrease intestinal permeability is another option. Furthermore, because the deamidation of gluten peptides by TG2 and the subsequent presentation by HLA-DQ2/8 initiates the adaptive immune responses, TG2 inhibitors and DQ2 blocking peptides seem to be an attractive possibility to prevent inflammation. Another promising alternative, especially for patients with refractory celiac disease, is directly targeting the immune cells either by lymphocyte blocking (anti–IL-15, anti-CCR9, anti-$\alpha 4\beta 7$) or tolerance induction.

transcription and translation at the beginning of endosperm development, whereas the high-molecular-weight glutenin gene promoters are not DNA methylated and would thus not be affected, theoretically preserving much of the baking quality.

Pretreatment of flours. Certain lactobacilli added to sourdough for fermentation are able to proteolyze the proline/glutamine-rich gluten peptides and thus decrease immunotoxicity.[204–206] Sourdough containing 30% fermented wheat flour plus a mixture of oat, millet, and buckwheat permits the production of bread with a texture comparable to that of regular wheat sourdough bread. A pilot study in 17 patients with celiac disease suggested that this sourdough bread was well tolerated.

Table 2. Novel Therapies for Celiac Disease

Target	Drug/modification	State of development	Reference
Intraluminal therapies			
Wheat varieties	(Ancient) wheat variants with low immunogenicity	Preclinical, tested biopsy specimens and gliadin-reactive T-cell lines	191, 193–197 198
	Genetically modified wheat variants or deletion lines of common wheat with lower immunogenecity		
Flour/dough	Pretreatment with lactobacilli	Clinical trial on 17 patients	207
	Transamidation of gliadin	Preclinical, tested on gliadin-reactive T-cell lines	209
Ingested gliadin peptides	Prolyl endopeptidases from Aspergillus niger	Phase 1 clinical trial (NCT00810654)	219, 220
	Sphingomonas capsulate in combination with (EP)-B2 from germinating barley	Phase 1 clinical trial (NCT00626184)	226, 227
	Intraluminal gliadin binding by polymers	Preclinical	228
	Gluten neutralizing cow's milk antibodies	Preclinical	
Transepithelial uptake			
Epithelial tight junctions	ZOT receptor antagonist AT1001	Phase 2b clinical trial (NCT00889473)	171
Dampening of the adaptive immune response			
TG2	Transglutaminase inhibitors	Preclinical, tested ex vivo on biopsy specimens	240, 241
	"Inhibitory" innate gluten peptides	Preclinical, tested on biopsy specimens and gliadin-reactive T-cell lines	242–249
HLA-DQ2	Blocking DQ2 analogues	Preclinical, tested on gliadin-reactive T-cell lines	251, 252, 256, 257
Immune modulators			
	Hookworm infection	Phase 2 clinical trial (NCT00671138)	264–266
	Gluten "vaccination" (Nexvax2)	Phase 1–2 clinical trial (NCT00879749)	262
Biologicals (systemic T-cell or cytokine blockers)			
Small intestine homing T cells	CCR9 antagonists (Ccx282-B, CCX025)	Phase 2 clinical trial planned (NCT00540657)	273
Gut homing T cells	Anti-integrin $\alpha4\beta7$ (LDP-02)	Phase 2 clinical trial for Crohn's disease (NCT00655135)	
Clonal IELs	Anti–IL-15 (AMG 714),	Phase 2 clinical trial for rheumatoid arthritis (NCT00433875)	277
	Anti-Jak3 (CP-690-550)		278
		Phase 2 clinical trial for rheumatoid arthritis, transplant rejection (NCT00550446, NCT00658359)	
Clonal intestinal T cells	Autologous bone marrow transplantation	Clinical trial on patients with EATL	132
	Mesenchymal stem cell transplantation (prochymal)	Phase 2 clinical trial for Crohn's disease (NCT00294112)	280
Mucosal destruction in refractory celiac disease	Anti–tumor necrosis factor α, anti–IFN-γ (HuZAF)	Case reports in celiac disease	285, 286
	Anti-CD52 (Alemtuzumab)	Phase 2 clinical trial for Crohn's disease (NCT00072943)	287
		Case reports in celiac disease	

NOTE. **Bolded** text is for subheading clarification only.

However, patients were only challenged for 2 days, which is much too short to draw final conclusions.[207]

Similarly, intrinsic proteases produced during germination of wheat, when the amino acids from the gluten storage proteins are being used for the growing plant, can degrade immunogenic T-cell epitopes. This opened the possibility that flour based on germinating wheat, barley, or rye may be used to create "nontoxic" cereal products for patients with celiac disease.[208] However, removal of all major storage proteins is expected to go hand in hand with loss of baking quality. Nonetheless, the germinating proteases are used for oral enzyme therapy (see the following text).

Another approach is to inactivate immunogenic gluten epitopes by exploiting the same substrate specificity of TG2 that generates more potent immunostimulatory

gluten peptides via deamidation.[209] Thus, incubation of gliadin with TG2 and lysine methyl ester leads to quantitative cross-link formation between the TG2 target sequences in gliadin and the terminal amino group of lysine methyl ester. These lysine-modified gliadins lost their affinity to bind to HLA-DQ2, which in turn abrogated IFN-γ production by intestinal T-cell lines derived from HLA-DQ2–positive patients with celiac disease. Furthermore, treatment of whole wheat flour with a low-molecular-weight microbial TG derived from *Streptomyces moboraensis* equally abrogated the stimulatory effect of the flour on gluten-reactive T-cell lines. Importantly, treatment with microbial TG improves loaf volume and crumb texture of breads.[210] This could make pretreatment of flour with microbial TG (and nontoxic lysine methyl ester) attractive for patients with celiac disease. Microbial TG is already applied by the food industry all over the world to improve doughs or the texture of foods in general.[210,211] However, a note of caution is necessary, because treatment of flour with microbial TG increased rather than decreased the stimulation of gliadin-specific T-cell lines.[212,213]

Future studies have to show how far these modifications can lead to cereal products that are largely devoid of immunogenic epitopes. Furthermore, the products must maintain the desired consistency and baking properties, large-scale industrial production must be cost effective, and issues such as the nutritional value of the products, the degree of removal of immunogenic epitopes, and the lack of, for example, de novo generated antigenic epitopes need to be addressed. Finally, the general acceptance of (truly genetically) modified cereal products may be low and their consumption may be mainly limited to the patients' households.

Oral enzyme therapy. In general, proteins reaching the intestinal lumen are digested by gastric pepsin and pancreatic proteases and further degraded by brush border enzymes to yield single amino acids, dipeptides, or tripeptides that are transported across the epithelial layer. However, the large quantity of proline residues,[214] especially in immunodominant gliadin peptides like the 33mer, causes them to be highly resistant to human digestive proteases.[68,215,216] Hence, one strategy to prevent those peptides from reaching the lamina propria has been to make use of prolyl endopeptidases (PEP) that are expressed in various microorganisms such as *Flavobacterium meningosepticum*, *Sphingomonas capsulata*, and *Myxococcus xanthus* and that are able to cleave the immunodominant proline-rich regions.[217–221] A pilot and safety study using PEP from *F meningosepticum* admixed to a daily drink with 5 g of gluten over 2 weeks could prevent fat malabsorption and symptoms in some patients with previously diet-controlled celiac disease.[167] However, neither the potency of the enzyme nor the sensitivity of the readouts (stool fat and D-xylose absorption) were considered sufficient to draw clear conclusions. The effectiveness of PEPs can be limited by restrictions on the length of their substrates,[222,223] their activity maximum at near-neutral pH, and the long time necessary to completely digest the gliadin peptides.[224,225] A reasonable approach is therefore the use of enzymes with a broader-activity spectrum and combination enzyme therapy. Thus, PEP from *Aspergillus niger* is active at acidic pH and has a higher specific activity than PEP from *F meningosepticum* to inactivate immunodominant gluten epitopes.[219,220] Furthermore, endoprotease B2, a glutamine-specific protease of germinating barley in combination with PEP from *S capsulata*, can efficiently break down whole wheat gluten in vitro and in a rat model in vivo, largely abrogating its immunogenic potential, as assessed with several gluten-specific T-cell lines.[226,227] Both enzymes are active and stable at acid pH and can therefore be administered as lyophilized powders or simple capsules or tablets.[227] Both *A niger* PEP and the endoprotease B2/PEP combination enzyme therapy are currently in phase 1 clinical studies (Table 1). As with most therapies discussed here, oral enzyme therapy will probably not be able to sufficiently degrade immunogenic epitopes of a normal daily gluten ingestion amounting to >13 g, but rather eliminate the detrimental effect of a few hundred milligrams to a few grams of gluten in patients with high gluten sensitivity or refractory celiac disease type 1.

Intraluminal binding of gluten peptides. This approach has been suggested in a study that used a copolymer of polyhydroxy methacrylate and polystyrene sulfonate to bind gliadin in a fairly specific manner.[228] The polymer blocked gliadin digestion to smaller immunogenic peptides and attenuated the gliadin-induced increase in intestinal permeability and T-cell activation in CD4 HLA-DQ8 transgenic mice. However, it is expected that many other nutrient proteins will interact with the polymer and limit its activity in patients with celiac disease.

Neutralizing gluten antibodies. Orally ingested IgG is highly resistant to gastric acidity, and roughly 50% of neutralizing activity survives when reaching the terminal ileum.[229] Cow's milk antibodies are easy and cheap to produce. Based on this rationale, large-scale production of gluten-neutralizing antibodies is attractive. Importantly, these antibodies can be considered a safe nutritional product, similar to milk products, which would not be subject to strict regulatory approval. A clinical phase 1 trial in the United States is expected.

Transepithelial Uptake

Inhibition of intestinal permeability. An increase of intestinal permeability via opening of the epithelial tight junctions appears to be an important contributor to the influx of gluten peptides into the subepithelial lamina propria, where the destructive adaptive T-cell response to gluten is triggered and maintained. *Vibrio cholerae* secretes the ZOT toxin that opens the intestinal

epithelial tight junctions via the 66-kilodalton ZOT receptor.[230,231] In addition, the injured epithelium of patients with celiac disease releases a paracrine protein product (zonulin) that acts similar to ZOT. An octapeptide (AT-1001) with homology to ZOT (or zonulin) was developed that blocks the ZOT/zonulin receptor and thus protects tight junctional integrity. A pilot study using AT-1001 in 14 patients with celiac disease in remission and 7 controls who were challenged with a single dose of gluten prevented the decrease in intestinal permeability and ameliorated peripheral blood mononuclear cell IFN-γ production and urinary secretion of nitric oxide (a marker of NO synthase activation and inflammation).[170] AT-1001 is currently the best-studied pharmacologic agent to treat patients with celiac disease. Thus, a phase 2 dose-escalation study (1, 4, and 8 mg daily) was performed in 184 patients in remission who were challenged with 3 × 0.9 g of daily gluten over 42 days. Although the primary end point (a significant decrease of the lactulose to mannitol ratio vs the placebo group) was not reached, patients treated with AT-1001 had a significantly improved symptom score, a less pronounced autoantibody response, and lower urinary nitrate excretion when compared with the placebo controls.[171] As with the previously described "glutenases," the effect of this approach alone will likely be limited. However, its combination with other treatments could be highly attractive.

Dampening of the Adaptive Immune Response

Transglutaminase inhibitors. The use of TG2 inhibitors has been hypothesized as a possible therapeutic approach, because inhibiting gliadin peptide deamidation could reduce their binding to HLA-DQ2 and HLA-DQ8 and thus their T-cell stimulatory capacity. Because the >7 known transglutaminases share a high degree of sequence similarity, especially in their catalytic center, inhibitors do not display unique selectivity for TG2. Inhibitors that target the transglutaminase cross-linking activity have been developed and mainly tested in vitro.[232–235] These are (1) competitive inhibitors (putrescine, spermidine, histamine, monodansyl cadaverin, cadaverine, 5-pentylamine, fluoresceine, cystamine, and cysteamine),[236] (2) reversible inhibitors (mainly guanosine triphosphate analogues),[237] and (3) irreversible inhibitors (iodoacetamide, 3-halo-4,5-dihydroisoxazoles, carbobenzyloxy-L-glutaminyl glycine derivatives, 6-diazo-5-oxo-norleucine, 2-[(2-oxopropyl)thio]imidazolium derivatives).[238,239] Cystamine and the 2-[(2-oxopropyl)thio]imidazolium inhibitors (L682777 or R283) have also been tested ex vivo in cultures of small intestinal biopsy specimens of patients with celiac disease, where they blunted T-cell stimulatory activity of gliadin peptides.[240,241] The approach of transglutaminase inhibition, although potential useful, is risky, because (1) transglutaminase fulfill many important functions in tissue homeostasis and inhibition of other transglutaminase is expected, (2) agents need to be designed and tested that are taken up by the intestine and do not reach the systemic circulation, and (3) even a complete inhibition of transglutaminase-mediated gluten deamidation will not eliminate all immunogenic epitopes, especially in children.[71] Of note, transglutaminase inhibitors based on a high affinity thiol binding group were recently developed that display 70- to 225-fold specificity for TG2 over TG1, TG3, TG6, and factor XIII when tested in vitro (Pasternack R, Hils M, Zedira Company, Darmstadt, Germany, personal communication, September 2009), raising hopes for increased safety of this approach.

Gluten peptides that down-regulate innate responses. An "innate inhibitory" decapeptide (sequence QQPQDAVQPF) was isolated by affinity chromatography and gel filtration from durum wheat and tested in various in vitro systems.[242–249] This peptide prevented agglutination of K562 erythroleukemic cells induced by PT-digested gliadin or α-gliadin p31-43[243,245] and protected Caco2 intestinal epithelial cells from apoptosis induced by gliadin.[246] The inhibitory effect was also present when lymphocytes[247] or duodenal biopsy specimens[249] from patients with celiac disease were challenged with PT-gliadin in vitro. The investigators postulated that the decapeptide induced a switch from a Th1 to a Th2 T-cell phenotype, because it down-regulated IFN-γ and up-regulated IL-10 production of intestinal T cells in patients with celiac disease.[248] Others tested modifications of "toxic" gliadin peptides to obtain "antagonistic" peptides.[182,250–252] Modification of the proline residues P38, P39, and P42 of α-gliadin p31-43 abrogated its pathogenicity as evaluated by morphometric analysis on duodenal biopsy specimens of patients with celiac disease, but their activity as antagonists of the wild-type peptide or total gluten was not studied.[250] Therefore, while serving as proof of principle, the application of single modified peptides is unlikely to yield therapeutic agents.

HLA-DQ2 inhibitors. Adaptive immunity in celiac disease is driven by presentation of gliadin peptides on HLA-DQ2 in the majority of patients with celiac disease, followed by activation of $CD4^+$ T cells that initiate and maintain the Th1 inflammatory response. Therefore, blocking DQ2 represents an attractive target to prevent immune activation. Similar approaches have already been investigated in other autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, or type 1 diabetes mellitus, although without demonstrating clinical benefit, mainly due to inefficient drug delivery.[253–255] In view of the accessibility of the small intestine via the oral route, drug delivery should be easier in celiac disease.

Based on gliadin peptides that drive adaptive immunity in celiac disease, several peptides with high affinity to HLA-DQ2 were designed by amino acid substitution, dimerization, or introduction of aldehyde groups. Modification of α2-gliadin p57-73 led to partial agonists that

significantly inhibited IFN-γ production by peripheral blood mononuclear cells from patients with celiac disease in the presence of the stimulatory unmodified peptide.[182] Furthermore, replacement of leucine L11 and L18 residues in the α-gliadin 33mer "superantigen" with sterically bulky groups retained high DQ2 affinity but decreased T-cell recognition.[252] Similar results were obtained using azidoproline-containing gluten peptides that per se were not able to activate T cells, although they could block the effect of a stimulatory α9-gliadin peptide only when used at high concentrations.[251] However, most of the modified peptides still showed agonistic effects when tested on gliadin-specific T-cell lines. Moreover, binding affinity for most of the peptides was not high enough to efficiently block access of stimulatory gliadin peptides to DQ2.[251,252,256,257] Furthermore, this approach poses other problems. First, while it is still not well known how intact gliadin peptides reach the lamina propria, this is even less clear for the modified peptides that may have to compete with the (luminal) gliadin peptides to reach their target cells. Second, side effects such as immunosuppression or hypersensitivity responses are potential safety concerns. Therefore, this ambitious approach will require significant work to develop a highly specific, high-affinity, nontoxic, and nonimmunogenic compound before testing in humans.

Immune Modulation and Induction of Tolerance to Gluten

The perhaps most attractive and causal treatment would be the restoration of tolerance to ingested gluten. That this is feasible is exemplified by the observation that (1) only one out of 30 carriers of the major predisposition for celiac disease (ie, HLA-DQ2 or HLA-DQ8) will develop celiac disease in their lifetime and (2) 20% of 61 subjects whose celiac disease was diagnosed in childhood and who remained on a gluten-free diet for several years did not develop celiac disease despite having resumed a normal gluten-containing diet in adolescence for an average of 10 years.[258] Induction of tolerance has been attempted by intranasal administration of gliadin peptides in gliadin-sensitized Balb/c or transgenic DQ8 mice, resulting in a decreased T-cell proliferative response to gliadin and a decrease in the production of inflammatory cytokines.[259–261]

Another strategy used 3 select immunogenic 16mer peptides derived from α-gliadin, ω-gliadin, and hordein that account for 60% of the overall gluten T-cell response to immunize gliadin-specific TCR/DQ2 transgenic mice via subcutaneous injections. This "gluten vaccination" suppressed $CD4^+$ T-cell proliferation and IL-2 and IFN-γ production and increased the expression of Treg markers by splenic $CD4^+$ cells in response to a gluten challenge.[262] A clinical study is on the way in Australia.

A simple, safe, and cost-effective method would be to down-regulate the proinflammatory (microbial) milieu of the small intestine in patients with celiac disease. Thus, addition of *Bifidobacterium* strains suppressed the proinflammatory effect of fecal extracts on peripheral blood mononuclear cells from patients with active celiac disease.[263] Clinical studies have not yet been performed. Another group from Australia has initiated a phase 1 clinical trial in patients with celiac disease using noninfectious larvae from the hookworm *Necator Americanus.* It is hoped that, similar to *Trichuris suis* therapy of inflammatory bowel disease, this treatment will skew the proinflammatory Th1 T-cell response to a less aggressive Th2 or a suppressive Treg response.[264–266]

Another approach used probiotic *Lactococcus lactis* that were engineered to secrete an immunogenic DQ8-restricted deamidated gliadin peptide. These bacteria were then administered to HLA-DQ8 transgenic mice after parenteral sensitization to the peptide, resulting in a diminished delayed-type hypersensitivity response, a diminished T-cell response to the peptide, and an increase in Foxp3-positive Tregs in the mesenteric lymph nodes.[267]

Therapies Targeted at Immune Cells

Most of these targeted therapies are currently used or evaluated in autoimmune diseases such as rheumatoid arthritis and/or inflammatory bowel disease. Although they are not justified to treat classic celiac disease, due to side effects and costs in view of a usually effective gluten-free diet, they hold promise in the treatment of refractory celiac disease and EATL. For most of these therapies, there exist case reports on their clinical utility at best (Table 1). The following discusses some of the targets and treatments that show promise for (refractory) celiac disease and EATL.

CCR9 and integrin α4β7 antagonists. Chemokines and chemokine receptors play an important role in the selective recruitment of leukocytes from the circulation to target tissues. Effector/memory T cells that home to the small intestine (ie, the intestinal segment affected by celiac disease) express both CCR9 and integrin α4β7. CCR9 mediates small intestinal homing via binding to chemokine ligand 25 (CCL25) that is secreted by the intestinal epithelium, and integrin α4β7 mediates attachment to the mucosal vascular addressin MadCam-1 on intestinal high venular endothelium.[268–270] Thus, increased CCR9 expression is found in Crohn's disease, both in intestinal and peripheral lymphocytes.[269] In celiac disease, discordant results have been obtained; augmented CCR9 expression was detected in peripheral lymphocytes,[269] whereas CCR9 protein levels were reduced in IELs and lamina propria lymphocytes of duodenal biopsy specimens and the decreased CCR9 expression was associated with activated peripheral blood mononuclear cells.[271] Blockage of CCR9/CCL25 improved histologic damage in early phases of a mouse model of spontaneous ileitis,[272] supporting the role of CCR9 as a possible ther-

apeutic target. Thus, CCX282-B, a CCR9 inhibitor, ameliorated the severity of ileitis in a tumor necrosis factor α–driven model of chronic ileitis,[273] and a phase 2 clinical trial in patients with moderate to severe Crohn's disease showed a reduction of the Crohn's Disease Activity Index in 61% of patients versus 47% for placebo.[274,275] A study to evaluate the effect of CCX282-B compared with placebo on the villous height/crypt depth ratio of small intestinal biopsy specimens taken from subjects with celiac disease before and after gluten exposure has been planned.[276] CCX025, a second oral CCR9 inhibitor, is currently in a phase 1 safety trial (ChemoCentryx, Mountain View, CA). A phase 2 clinical trial has been planned, but the inhibitors are currently on hold for celiac disease. Similarly, the $\alpha 4\beta 7$ integrin blocking antibody LDP-02 is used in a phase 2 clinical trial for Crohn's disease (NCT00655135), but no trial in celiac disease has yet been initiated. The overall benefit of blocking lymphocyte homing to the small intestine in celiac disease is not clear, because beneficial immunosuppressive Tregs are equally inhibited.

IL-15 antagonists. The central role of IL-15 in the pathogenesis of (refractory) celiac disease has been highlighted in this review. IL-15–blocking antibodies have been tested in patients with rheumatoid arthritis.[277] Furthermore, an inhibitor of the downstream Jak3 signal transducer is currently being tested in phase 2 clinical trials for rheumatoid arthritis, transplant rejection, psoriasis, and inflammatory bowel disease.[278] Much hope has been invested in anti–IL-15 therapy, especially for refractory celiac disease type 2 and EATL in which the expansion of malignant lymphocytes appears to be driven by IL-15, but industry has so far been reluctant to support a clinical trial.

Bone marrow transplantation. Autologous bone marrow transplantation has been used to induce remission in patients with EATL.[132] Although remissions have been achieved, patients have experienced relapses due to residual cells that reside in the transplanted autologous bone marrow. Therefore, heterologous bone marrow transplantation using cells from unaffected donors is more promising but also more risky. No studies using heterologous bone marrow transplantation have yet been reported.

Mesenchymal stem cell therapy. A novel modality is the infusion of mesenchymal stem cells.[279] Mesenchymal stem cells differentiate in vitro and in vivo into multiple mesodermal tissues, including bone, cartilage, adipose tissue, tendon, ligament, or even muscle.[280] These cells can be produced in large quantities ex vivo from human donors. Importantly, they have low immunogenicity due to the lack of HLA class I or II and of costimulatory molecules.[281] Mesenchymal stem cells can therefore be infused safely into allogeneic recipients. Mesenchymal stem cells preferentially home to sites of organ damage, where they suppress lymphocyte proliferation.[282–284] Clinical studies (Prochymal; Osiris, Columbus, MD) are ongoing in numerous inflammatory and degenerative diseases showing benefit in severe (intestinal) graft-versus-host disease and therapy-resistant Crohn's disease.[280] It is conceivable that mesenchymal stem cell infusion can dampen or even abrogate the immune response to gluten in patients with celiac disease and perhaps in patients with refractory celiac disease or EATL. A clinical trial is planned.

Conclusions

Due to advanced understanding of its pathogenesis, numerous therapeutic strategies have been devised to treat celiac disease. With further advances in the development of preclinical models and better noninvasive activity markers, clinical validation of many of these therapies is anticipated in the next years. Of particular interest are (1) immune-based treatments that induce oral tolerance to gluten and are thus curative and (2) combination therapies that increase efficacy while at the same time having reduced side effects. The advances in celiac disease will also spawn therapeutic developments for other immune-mediated disorders such as inflammatory bowel disease or autoimmune disease of other organs for which celiac disease can serve as a well-defined model disease.

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Conflicts of interest

The authors disclose no conflicts.

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