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Celiac disease

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Abstract

With a prevalence of around 1%, celiac disease is one of the most common autoimmune disorders. It is an inflammatory disease that is triggered by the ingestion of gluten in genetically predisposed individuals. The symptoms and the severity of the disease vary hugely, reflecting its systemic nature. Classically, the villi and the crypt are destroyed in the small intestine, which leads to malabsorption of nutrients and malnutrition. However, many other gluten-dependent symptoms may be present, often without the 'classic' enteropathies. These include skin disorders, neuropathies, complications of the hepatic, renal and endocrine systems, and malignancies. This review focuses on the risk factors and complications of celiac disease, with a brief overview of the immunopathology and potential strategies for developing therapeutic agents.

Introduction

Celiac disease (CD) is an inflammatory disorder that is precipitated by the ingestion of gluten-containing foods in genetically predisposed individuals (1-4). In the classical form of the disease, inflammation and autodestruction of the mucosa in the small intestine cause the loss of the villus and crypt structure, which leads to reduced food digestion and absorption of vitamins and nutrients (Fig. 1). The symptoms include chronic diarrhea, abdominal discomfort, fatigue and symptoms of malnutrition such as iron deficiency anemia, osteoporosis, and in children, stunted growth. However, those with the classical symptoms represent only a small proportion of the total who test positive for serum autoantibodies (anti-endomysium and anti-tissue transglutaminase) that are indicative of active CD. Seropositive individuals may be asymptomatic, perhaps representing a group of patients whose disease is latent, while others present with extraintestinal manifestations, including hepatic, renal, nervous system, dermatological and autoimmune endocrine disorders, as well as malignancies, often in the absence of the classical gastrointestinal symptoms. For example, some patients develop dermatitis herpetiformis, an extremely itchy skin rash, have histological modifications in the small intestine and yet have no gastrointestinal symptoms. The only recommended treatment is a lifelong gluten-free diet, which is effective in most cases. The prevalence of CD is estimated to be around 1% of the Northern European and U.S. populations (5), and this figure is probably not dissimilar worldwide (6). The risk increases among first-degree (up to 20%) and second-degree relatives (about 2.5%), indicating a strong familial component (5).

Risk factors

Two factors are absolutely required to initiate the autoimmune reaction: one is the presence of the major histocompatibility class (MHC) II protein HLA-DQ2 or -DQ8 (an inherited predisposition) and the other is the ingestion of gluten-containing foods such as wheat, barley or rye. However, about 30% of the general population carry the gene for either HLA-DQ2 or -DQ8 and the majority are probably exposed to dietary gluten (in the U.S. at least, where most studies have been conducted), yet only 1% of the population actually develop the disease. This means that, although necessary, possession of HLA-DQ2/8 plus ingestion of gluten is not sufficient for the disease to develop, and other (poorly characterized) genetic or environmental factors must be involved (2).

Approximately 90-95% of CD patients carry the alleles HLA-DQB1*02 and HLA-DQA1*05 located at 6p21.3, which encode the HLA-DQ2 heterodimer. The remaining 5-10% of patients carry the alleles HLA-DQB1*0302 and HLA-DQA1*03, which encode the HLA-DQ8 heterodimer. These statistics show that possession of these genes is absolutely required for development of the disease. These HLA risk alleles are estimated to contribute about 40% to the total genetic risk of CD, indicating that other genes are involved in the pathogenesis of the disease.

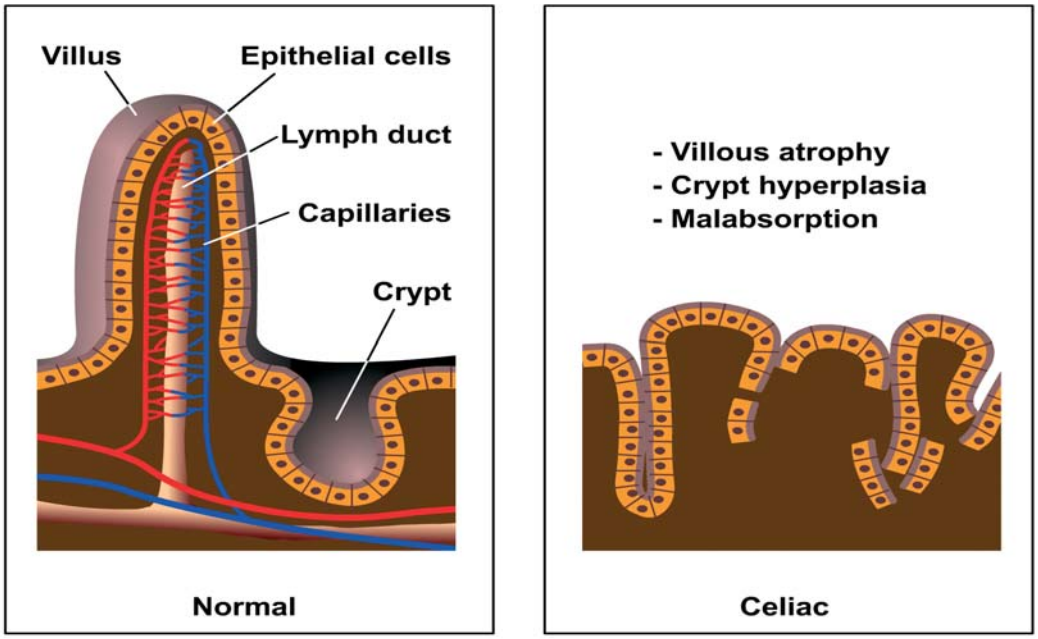

Fig. 1. Small intestinal mucosal injury in celiac disease. Left-hand side: normal mucosal and villous architecture. Right-hand side: loss of villous structure and crypt hyperplasia, as seen in patients with severe forms of celiac disease.

There is a gene dose effect and the number and quality of HLA-DQ molecules dictate the risk and magnitude of the response to dietary gluten (2, 4, 7, 8).

The concordance rate for CD in monozygotic twins is 83%, and in dizygotic twins it is 17%, irrespective of the sex or HLA genotype of the co-twin. This shows that genetics makes a strong contribution to the disease and that monozygotic twins share non-HLA susceptibility genes that are not always present in the dizygotic twins (9, 10). Other candidate regions have been identified, although their individual contributions to the disease are weak in comparison to that of HLA-DQ, and the findings have been inconsistent. One possible explanation is that the remaining genetic component might be made up of a summation of several low-frequency variants in different regions of the genome, each individually contributing a moderate effect. The non-HLA regions that have most consistently been linked to CD include 5q31-33 (11), 2q33 harboring the CTLA4 gene, a co-stimulatory molecule expressed on activated T-cells (12), 19p13.1 harboring the gene for myosin IXB involved in cytoskeletal rearrangement in the intestine (13) and possibly in the maturation of antigen-presenting cells (APCs) in inflammatory lesions, and 15q11-13 (14). A large genome-wide association study has also identified an association with 4q26-27, harboring IL-2 and IL-21 (15).

Dietary intake of the gliadins and glutenins (the components of gluten) found in wheat, the hordeins found in barley, the secalins found in rye, and perhaps the avenins found in oats, is required to activate the disease. A gluten-free diet reverses the symptoms and tissue damage, and a gluten re-challenge exacerbates the condition. The α-gliadin peptide fragments p31-49 and p31-43, as well as a 33-mer deaminated fragment of α-gliadin, are three highly immunogenic peptides that have been much studied, although many other gluten fragments are expected to promote similar responses (16, 17). In this article, these proteins are collectively referred to as 'gluten'.

Other environmental risk factors include surgery, pregnancy, childbirth, severe emotional stress (1, 3), interferon alfa treatment for HCV infection (18) and repeated rotavirus infection in childhood (19).

The peak period for diagnosis is between the fourth and sixth decades of life, although symptoms often appear between 6 and 18 months of age, coinciding with the introduction of cereals into the diet and weaning (20, 21). There is a 1:1 sex ratio in children. In adults there is a female predominance of 3:1 (21).

Disease pathology

Increased permeability of the small intestine allows gluten to enter the lamina propria (the layer of loose connective tissue just below the intestinal epithelium), where it interacts with tissue transglutaminase (TG2 or tTG) and the innate and adaptive immune systems to initiate an autoimmune response. Inappropriate responses include the development of autoantibodies such as anti-TG2 and anti-actin antibodies, maturation of $CD4^+$ Th1 helper cells that produce inflammatory cytokines and of $CD8^+$ cytotoxic T-cells that are directed against the epithelial cells of the intestinal mucosa (Fig. 2). Together, these reactions cause the remodeling of the mucosa and the nutrient malabsorption seen in classical CD. The pathology of the extraintestinal manifestations of CD is less well understood, although systemic autoantibodies are thought to play a role (2, 22).

Intestinal permeability

Glutens are rich in the amino acids proline and glutamine, which makes them resistant to intestinal proteases, and peptides of up to 50 amino acids may persist in the lumen of the small intestine (23). Under normal circumstances, the intestinal epithelia do not allow undigested macromolecules such as gluten peptides into the lamina propria, but in untreated patients with active CD, intestinal permeability is increased and the gluten peptides are able to pass through to the lamina propria relatively intact (24, 25).

The mechanistic basis for increased intestinal permeability in CD is poorly understood, but several mechanisms have been implicated: 1) incomplete digestion of gluten by enzymes located in the brush border of differentiated enterocytes (26); 2) increased intestinal production of nitrite induced by enteric glial-derived S100B protein (27); 3) increased trafficking via the epithelial exosome (vesicle)-mediated pathway, which is also coupled to the increased activation of T-cells in the lamina propria (28, 29); 4) gluten-mediated upregulation of zonulin, resulting in remodeling of actin at the tight junctions between the epithelial cells and increased intestinal permeability via the paracellular pathway (30); and 5) interactions between gluten and structural proteins in the small intestine, such as actin, which might cause these proteins to behave as autoantigens, leading to remodeling of the intestinal epithelia (31).

Likewise, it is not clear if intestinal permeability to gluten is a cause or a consequence of the disease. The possible cases where it might be a cause include:

- An intrinsic difference in intestinal permeability may predispose some individuals to the disease. Variants of the *MYO9B* gene have been associated with an increased risk of CD in some populations (13). The myosin IXB protein is involved in remodeling of the cytoskeleton at the tight junctions and impairment of its activity has been postulated to increase intestinal permeability. It should be noted, however, that the association between *MYO9B* variants and CD has been demonstrated in some studies (13, 32), but not in others (*e.g.* [33]).
- Repeated rotavirus infections have been associated with an increased incidence of CD in children (19). The virus might serve as a trigger for CD by initiating intesti-

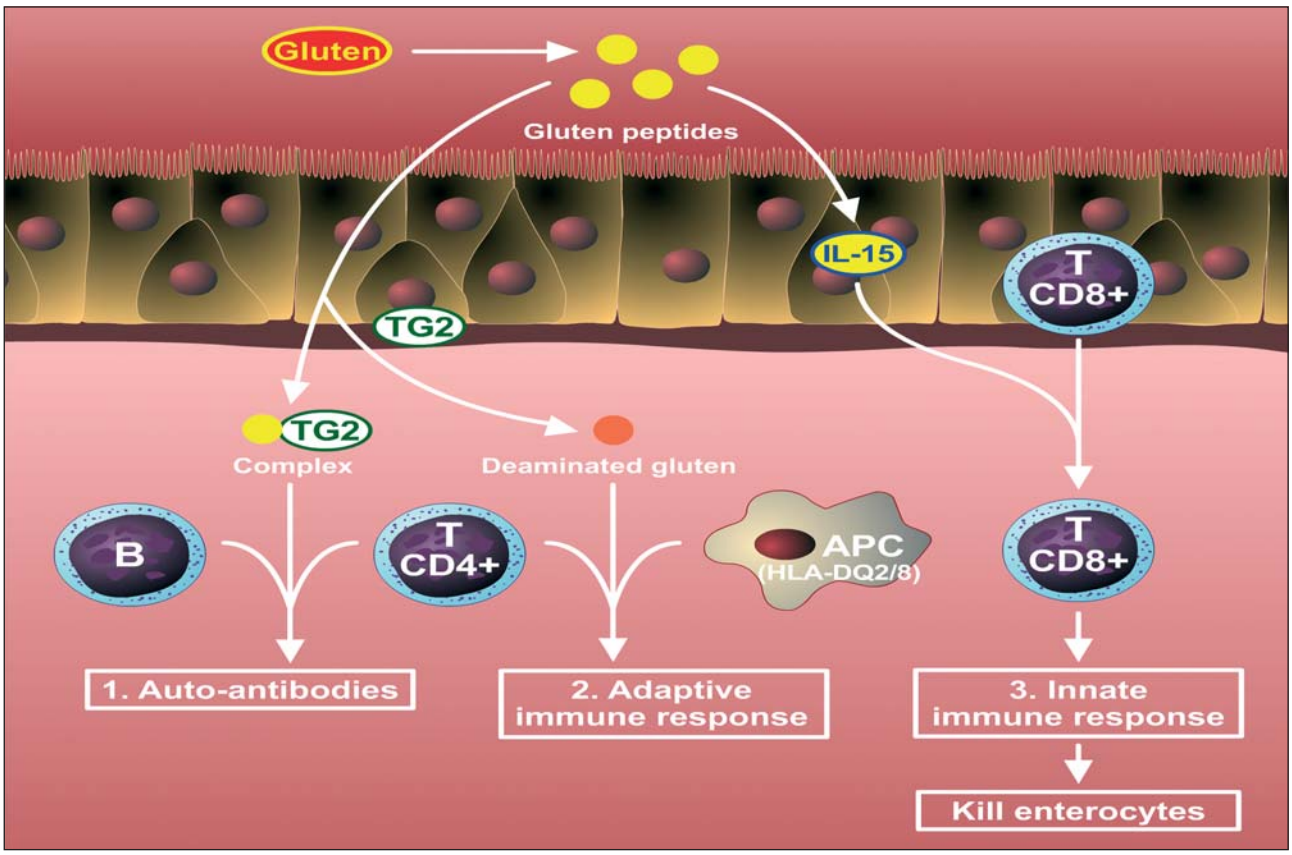

Fig. 2. A model for the immunopathology of celiac disease in the small intestine. 1) Autoantibodies against tissue transglutaminase (TG2) are produced by B-cells when stimulated by stable gluten-TG2 complexes. 2) Gluten peptides that have been deaminated by TG2 bind to HLA-DQ2/8 molecules on antigen-presenting cells (APCs) and activate gluten-reactive $CD4^+$ T-cells to trigger an adaptive immune response. 3) Gluten peptides trigger an innate immune response by stimulating IL-15 expression by epithelial cells. This induces intraepithelial $CD8^+$ T-cells to transform into natural killer (NK) cells that attack enterocytes.

nal inflammation that both increases intestinal permeability and potentiates the immune system to an anti-gluten reaction in susceptible individuals (16).

Tissue transglutaminase

Once in the lamina propria, key glutamine residues on the gluten peptides may be deaminated by TG2 to generate highly immunogenic derivatives, such as the deaminated 33-mer α-gliadin peptide (34). Besides increasing the immunogenicity of gluten peptides by deamination, the TG2-gluten interaction may contribute to other processes of CD, such as activation of the innate immune response, re-arrangement of actin, epithelial phosphorylation and apoptosis, and may perhaps also account for some of the extraintestinal manifestations of the disease (35, 36). Anti-TG2 antibodies are one of the hallmarks of CD and they are thought to be produced as a consequence of the formation of relatively stable TG2-gluten complexes, although whether they contribute to or are a consequence of the disease pathology is not clear (37, 38).

The immune response

CD is characterized by an increased number of T-cells in the lamina propria. Gluten interacts with components of both the innate and adaptive immune systems and the two cross-react with each other to trigger the inflammatory reactions and inappropriate T-cell responses against self-tissue (17).

The left-handed polyproline helical configuration of gluten favors binding to the peptide groove of the HLA-DQ2/8 heterodimer, and deaminated gluten has even stronger binding (23, 34, 39, 40). The gluten-HLA complex is presented on the surface of APCs, which then interact with gluten-receptive $CD4^+$ T helper cells and cause their activation through the Th1 pathway. This skew to Th1 cells results in an overproduction of the proinflammatory cytokine interferon gamma (IFN-γ), the release of metalloproteinases and an exaggerated immune response (see Fig. 2). In addition to its proinflammatory properties, IFN-γ exacerbates the adaptive immune response by enhancing the expression of the genes for HLA-DQ2/8 and metalloproteinases and the proliferation of the gluten-reactive $CD4^+$ cells in the lamina propria, and directs T-cell maturation through the Th1 pathway (17).

Gluten peptides, including the α-gliadin peptide fragment p31-43, stimulate intestinal epithelial cells to produce proinflammatory cytokines such as IL-15 (41). IL-15 in turn stimulates the transformation of intraepithelial $CD8^+$ T-cells to natural killer (NK) cells that express the surface epitope NKG2D. It also promotes increased levels of MIC (a ligand of NKG2D) on the surface of epithelial cells, which labels them as targets for the $CD8^+$ NK cells via MIC-NKG2D interactions, thus contributing to the villous atrophy. Additionally, IL-15 promotes various other stress response reactions in the intestine, including upregulation of cyclooxygenase type 2 (COX-2) activity, the production of proinflammatory cytokines such as IFN-γ and tumor necrosis factor-α (TNF-α), and the emergence of T-cell clonal proliferations that can lead to lymphomas (42-44). Both gluten and IL-15 have been shown to stimulate the maturation of dendritic cells. These cells play a role in antigen presentation to $CD4^+$ T-cells and provide a point of 'cross-talk' between the innate and adaptive immune systems (29, 45).

The requirement for the HLA-DQ2/8 proteins suggests that the adaptive response is a necessary component of CD. However, the fact that most people who possess HLA-DQ2/8 do not develop CD despite ingestion of wheat, and that the severity of symptoms and histopathology vary widely in those who do develop the disease, implies that an HLA-mediated adaptive response alone is not sufficient. Conversely, gluten-dependent IL-15 secretion by enterocytes and the maturation of $CD8^+$ T-cells do not take place in individuals without the HLA susceptibility alleles (46). For these reasons, it is thought that an interplay between the adaptive and innate immune responses may be required for the full expression of the disease and that the proinflammatory cytokines IFN-γ and IL-15 play a crucial role in this interaction (2, 47).

Refractory sprue is characterized by severe malnutrition or villous atrophy that does not improve after 6 months of a strict gluten-free diet. It affects around 8% of the CD population and typically occurs in older patients (48). Patients with this condition can be divided into two subgroups: those with type I refractory disease have large numbers of normal intraepithelial lymphocytes, and those with type II refractory disease have clonal expansion of immature intraepithelial lymphocytes which lack the normal T-cell markers. This clonal expansion is driven by IL-15 released from stressed epithelial cells in a gluten-independent manner and usually precedes the development of a lymphoma known as enteropathy-associated T-cell lymphoma (EATL) (49).

Associated diseases

CD is a systemic disorder affecting many organs and tissues. This is reflected in the large number of disorders associated with CD (1, 3, 50).

Gastrointestinal

CD is over-represented in patients with other gastrointestinal disorders, including inflammatory bowel disease, ulcerative colitis, Crohn's disease, mouth ulcers (1) and both lymphocytic and collagenous microscopic colitis (51). CD is negatively associated with perforated appendicitis (52). It is not clear to what extent these disease associations can be attributed to common underlying pathologies or to an initial underdiagnosis of CD in these other patient groups.

Nutrient malabsorption

Changes in the small intestine make it less able to absorb nutrients, vitamins and minerals. General symp-

toms related to nutrient malabsorption include fatigue, weight loss, missed menstrual periods, failure to thrive (children), delayed puberty, dental enamel hyperplasia and possibly congenital malformations (1, 3). With regard to the latter, conflicting results have been obtained (53, 54), and if a connection does exist, it is probably secondary to malnutrition during pregnancy.

One of the more common hematological consequences of malnutrition in CD is iron deficiency anemia; up to 15% of patients with symptomatic iron deficiency anemia have CD, as well as 6% of those with asymptomatic iron deficiency anemia (5). The possible causes of this association are malabsorption of iron, vitamin B_{12} and folic acid, as well as occult blood loss from the gastrointestinal tract. Increased inflammatory reactions have been associated with anemia in CD patients (55). Other hematological presentations include megaloblastic anemia and abnormal coagulation with a tendency to bleed, caused by malabsorption of vitamin K. Hyposplenism is found in 20-80% of CD patients and has been linked to an increased risk of bacterial infections, autoimmune and thrombotic diseases and solid tumors (56, 57).

Bone diseases in CD patients may result from the malabsorption of calcium and vitamin D, from elevated levels of the inflammatory cytokines IL-1β and IL-6, which influence bone formation and resorption (58), and in some cases chronic renal failure can contribute to bone diseases by failing to convert vitamin D to its active form, and this in turn leads to hypocalcemia. Vitamin D and calcium deficiencies lead to a reduced bone mineral density and an increased risk of osteopenia, osteoporosis and fractures in CD patients (5, 59, 60). Compensatory or secondary hyperparathyroidism is caused by persistent low blood calcium levels and this condition leads to bone diseases, elevated blood pressure and immune and neurological problems (61).

Extraintestinal

While some of the manifestations of CD can be explained by malnutrition, many cannot, reflecting the systemic nature of the disease (62). CD is associated with an increased risk of kidney diseases including glomerulonephritis (any form), dialysis and kidney transplantation in adults (63). Chronic kidney failure may also lead to secondary hyperparathyroidism and attendant problems.

The most common liver diseases linked to CD include elevated aminotransferase levels of unknown cause (present in around 50% of CD patients, and CD is likewise over-represented in those with unexplained transaminasemia) and autoimmune hepatitis (3). Other liver conditions that have been linked to CD include acute and chronic liver disease, hepatic steatosis, liver cirrhosis, primary sclerosing cholangitis, nonalcoholic fatty liver disease and liver failure (64). The elevated incidence of liver disease in CD patients is thought to be caused by the increase in intestinal permeability, which allows greater levels of toxins and pathogens to pass into the bloodstream, leading to increased stress on the liver.

The prevalence of cardiac diseases such as idiopathic dilated cardiomyopathy and autoimmune myocarditis is generally increased in CD patients, perhaps secondary to some of the other manifestations such as diabetes (65).

Respiratory diseases are a major cause of death in CD, mainly attributable to lung abscesses caused by previous pulmonary infections (66). Not every case of lung abscess can be attributed to lung infections, however, and deficiencies in the lymphoid and reticular endothelial systems linked to hyposplenism have been suggested as a possible cause of respiratory disease in CD patients (58).

CD is linked to a number of chromosomal and developmental disorders, including Down's syndrome, where the prevalence of CD is 5-8%, Turner's syndrome, where CD is present in 6.3%, Williams syndrome and autistic disorder (3). The connection between CD and Down's syndrome is thought not to involve shared HLA susceptibility genes (67).

Neurological complications are seen in approximately 6-10% of CD patients and occur with or without intestinal pathologies (68), perhaps secondary to immune activity against neuronal antigens (69). The most common complications include cerebellar ataxia, migraine and epilepsy; CD also increases the risk of depression around 2-fold (70). Other neurological complications of CD include schizophrenia, epilepsy with cerebellar calcification, dementia, cerebral vasculitis, brainstem encephalitis, encephalopathies, Huntington's disease, myopathy and multiple sclerosis (68). Peripheral neuropathies have been associated with CD in some studies, but a meta-analysis suggested no such link (71).

Reproductive complications have been associated with CD, including unexplained infertility in women (3). An increased risk for pregnancy-related problems, such as miscarriages and congenital malformations, has been reported, although the evidence is controversial (53, 54). These conditions, along with missed menstrual periods, are thought to be related to malnutrition in CD patients.

Dermatitis herpetiformis is an extremely itchy, blistering skin rash localized mostly on the articular joints. The condition affects around 25% of CD patients and is considered a variant of CD because they share the same HLA susceptibility alleles. Histological changes in the small intestine are often present in both conditions, although gastrointestinal symptoms tend not to be present with dermatitis herpetiformis (72). IgA antibodies and anti-epidermal transglutaminase autoantibodies may play a role in the pathogenesis of this condition (73). Other dermatological complications of CD include alopecia areata, psoriasis and vitiligo (72).

Infectious diseases, such as pneumococcal sepsis (56) and tuberculosis (74), occur at an increased frequency in CD patients. The spleen is responsible for the production of IgM memory B-cells that are required to clear infections caused by encapsulated bacteria such as pneumococci. The increased risk of hyposplenism in CD patients may, at least in part, lie behind this increased risk of infection (56, 57).

CD is co-morbid with several autoimmune endocrine disorders (about 14% of CD patients have autoimmune disorders compared to 2.8% of the nonceliac population [75]), many of which are connected through shared HLA susceptibility genes and increased serum levels of anti-actin antibodies.

Type 1 diabetes occurs in 1-7% of patients with CD and CD is present in 2-5% of adults and 3-8% of children with type 1 diabetes (5, 76, 77). These two disorders appear to be connected through many mechanisms, including shared HLA susceptibility genes (78), increased zonulin-mediated intestinal permeability (79), increased levels of TNF-α (78), recruitment of lymphocytes to the mucosa upon exposure to dietary gluten (80), and possibly through increased activity of TG2 (81). However, there are conflicting results as to whether dietary gluten induces type 1 diabetes (75, 82, 83) and there is little evidence that the diabetic symptoms in patients with type 1 diabetes improve with a gluten-free diet (84).

Autoimmune thyroid disease (hyper- or hypothyroidism) occurs in around 5% of CD patients, and the prevalence of CD in patients with autoimmune thyroiditis is 3% (1, 3). These diseases are probably not connected through HLA susceptibility genes (85), although they are both associated with increased levels of liver transaminases (86).

Addison's disease (chronic adrenal insufficiency) and Sjögren's syndrome (an autoimmune disease of the tear and salivary glands) have both been connected with CD (87, 88).

Furthermore, CD is co-morbid with other autoimmune disorders, such as selective immunoglobulin A (IgA) deficiency (2% of CD patients have IgA deficiency) and IgM deficiency, which increases the risk of hyposplenism and infections. CD patients are also at increased risk of systemic lupus erythematosus, collagen vascular disease, rheumatoid arthritis, autoimmune hepatitis and primary biliary cirrhosis. In many of these cases, it is not clear whether these autoimmune disorders share a common predisposition to CD, or whether CD itself precipitates the disorders (3, 4, 82).

The risk of death from malignancy in CD patients is 2.6-fold higher than in the general population, although this figure appears to be lower in some of the more recent studies, perhaps due to improved diagnosis of CD and better compliance with diet (3, 21, 89, 90). Of particular note, non-Hodgkin's lymphomas, particularly the T-cell, lymphoplasmacytic, diffuse large B-cell and mantle cell types, account for around two-thirds of all malignancies in CD patients (90).

Management

The only accepted treatment is a lifelong gluten-free diet; it has few risks and is effective in most patients. Symptoms in adults usually start to improve from 2 weeks after initiation of the diet and continue to improve over the next 2-5 years, although recovery may not always be complete, especially when CD is diagnosed late in life. In children, the improvements are often seen rapidly and tend to be complete. Persistent symptoms are almost always caused by continued intake of gluten, even if unwittingly. Nutrient supplementation is sometimes given to those newly diagnosed with CD and initiating a gluten-free diet, and the need for supplements disappears once the patient has recovered. Support groups, the physician and a dietician form an important part of managing CD, both for the patient and the family (3, 91, 92).

Epidemiological studies have shown that breastfeeding during the introduction of dietary gluten is associated with a reduced risk of developing CD in childhood (93).

Some of the complications associated with CD are managed with pharmacotherapies in conjunction with a gluten-free diet. Topical dapsone can help to resolve the itching and rash associated with dermatitis herpetiformis. Patients with bone fragility fractures or with a bone mineral density of < 2.5 standard deviations below the average for a healthy young adult may benefit from hormone replacement therapy, a bisphosphonate or calcitonin. CD patients with hyposplenism are immunized with pneumococcal conjugate vaccine. Some patients with type I refractory CD respond to corticosteroids such as prednisone and oral budesonide and to immunosuppressants such as azathioprine and ciclosporin (1, 3, 94).

Therapeutics in the pipeline

A gluten-free diet is safe, effective and relatively low-cost, and any new pharmacotherapy would have to be weighed against this benchmark. However, many patients express a desire for pharmacological treatment because of difficulty with adherence to a gluten-free diet and for social reasons. There is a great deal of interest in developing therapies for patients with CD.

Probactrix® is a live, nonpathogenic strain of *Escherichia coli* under development by BioBalance, a wholly owned subsidiary of New York Health Care, for use in the dietary management of patients with various gastrointestinal disorders. An IND was filed with the FDA in 2005 to begin clinical evaluation of the agent in patients with CD, although links to BioBalance's web site are no longer active (95).

AT-1001 is a zonulin receptor antagonist that prevents zonulin-mediated opening of the epithelial tight junction. The agent originated from a collaboration between Alba Therapeutics and the University of Maryland. Results from a randomized, double-blind, placebo-controlled phase IIa trial in 86 CD patients showed that the agent reduced intestinal permeability and improved patient symptoms and outcomes in response to a gluten challenge. There were no severe adverse events. A phase IIb trial was recently initiated (96, 97).

Another potential therapeutic approach is the use of prolyl endopeptidases to digest the gluten before it reaches the small intestine. This might be achieved either by ingestion of the proteases along with gluten-containing food to aid digestion (98), or by protease treatment of gluten-containing foods before they are eaten (99).

Vaccines for autoimmune disorders are based on the idea that specific epitopes of the autoantigen might be found that will redress the pathogenic Th1 pathways in the adaptive immune system and promote tolerance. For example, glutamic acid decarboxylase (GAD) is the autoantigen that promotes the autoimmune destruction of β-cells in diabetes. The recombinant human GAD65 epitope induces maturation of a subset of regulatory T-cells that suppress the pathogenic maturation of $CD4^+$ and $CD8^+$ cells involved in the autoimmune process. The treatment was found to be safe and effective in a phase II study in patients with type 1 diabetes (100). In the case of CD, a gluten/HLA-DQ2/8/TG2-derived epitope might be expected to produce similar results (101).

Recent advances in understanding the immunopathogenesis of CD have identified several potential targets, including IL-15 (46), p38 mitogen-activated protein (MAP) kinase (46), the interaction between HLA-DQ2 and $CD4^+$ T-cells (102), TG2 (103), $CCR9^+$ T-cells (104), the NKG2D-MIRA interaction, IFN-γ (105) and IL-10 (106).

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