

Coeliac disease

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Coeliac disease is a chronic inflammatory disorder of the small bowel induced in genetically susceptible people by the irritant gluten and possibly other environmental cofactors. The disorder is characterised by a diverse clinical heterogeneity that ranges from asymptomatic to severely symptomatic, and it manifests with frank malabsorption, an increased morbidity attributable to the frequent association with autoimmune disorders and increased mortality resulting from the emergence of T-cell clonal proliferations that predispose the patient to enteropathy-type T-cell lymphoma. Our understanding of the molecular basis for this disorder has improved and enabled the identification of targets for new therapies, although a strict gluten-free diet remains the mainstay of safe and effective treatment. In this Seminar we critically reassess the clinical and diagnostic aspects of this disease and new perspectives in its pathogenesis and treatment.

Introduction

Coeliac disease is a chronic inflammatory disease characterised by flattened villi on the small bowel mucosa, and is induced in genetically susceptible people by the ingestion of proline-rich and glutamine-rich proteins in wheat, rye, and barley. Researchers postulate that the condition first developed after the last ice age in the fertile crescent of the Middle East with the cultivation of grains, and the first description dates from the 1st and 2nd centuries CE.¹ Coeliac disease has a diverse clinical heterogeneity, and increases both morbidity and mortality. However, knowledge of many aspects of this disorder is inadequate—even in the academic specialty setting.²

Epidemiology

The accuracy of estimates of the true prevalence of coeliac disease has been substantially improved by the increased reliability of serological tests—namely for IgA anti-gliadin antibodies, initially, then for antiendomysial antibodies and IgA antihuman tissue transglutaminase (hTTG) antibodies. In large population samples, these tests enable screening for people who need a biopsy to confirm intestinal coeliac lesions. By means of this approach, the prevalence of biopsy-proven coeliac disease in Finnish and Italian schoolchildren was reported to be 1:99 and 1:106, respectively.^{3,4} Similar rates of seroprevalence have been reported in adult populations in the UK (1:87)⁵ and USA (1:105),⁶ thus drawing attention to the very high frequency of the disease in white people. The disorder is less common in Hispanic Americans⁷ and is thought to be rare in central Africa and east Asia. The highest

rate of antiendomysial antibody positivity (5·6%) has been reported in Saharawi children.⁸

Causation

Coeliac disease develops as a consequence of the encounter between an environmental trigger and a genetically predisposed host, with the possible participation of other environmental cofactors (figure 1).

Triggers

Gluten is a rubbery mass that consists of storage proteins that remain after starch is washed from wheat-flour dough. These proteins have different solubilities in alcohol–water solutions and, thus, can be roughly separated into two fractions—gliadins and glutenins. Gluten proteins have a complex chemistry and are responsible for the baking properties of wheat—water absorption capacity, cohesivity, viscosity, and dough elasticity.⁹ Analysis of gliadin has identified more than a hundred components that can be grouped into four main types (ω 5-, ω 1,2-, α / β -, γ -gliadins). The immunogenicity and toxicity of several gliadin epitopes

Search strategy and selection criteria

We searched Medline using the medical subject heading (MeSH) terms “coeliac disease” and “celiac disease” for articles published between January, 1998, and January, 2009, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of review articles on coeliac disease.

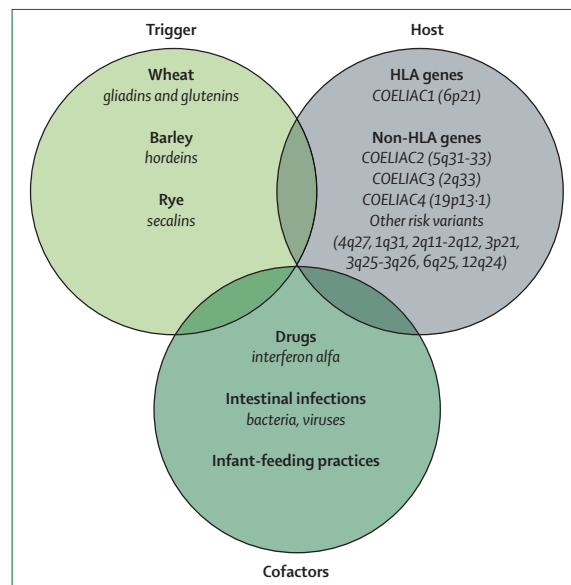


Figure 1: Causative factors in coeliac disease

has been established.¹⁰ A distinction exists between a peptide being immunogenic or toxic. Lymphocyte-based systems are used to assess immunostimulatory properties and, so far, all peptides that are immunostimulatory in vitro are toxic when tested in vivo. However, ex-vivo or in-vivo experiments are needed to confirm toxicity. Investigators have, however, identified a peptide that is toxic both in people¹¹ and to coeliac small-intestinal explants¹² that does not stimulate coeliac gluten-sensitive small-intestinal T cells in vitro. Thus, an absence of in-vitro stimulatory capacity of a peptide does not exclude it from causing toxic effects in patients.

Glutenins can be divided into groups of high molecular weight and low molecular weight. Immunogenicity¹³ and toxicity¹⁴ in the high-weight group have been shown. Storage proteins (prolamines), with a similar aminoacid composition to the gliadin fractions of wheat, have been identified in barley (hordeins) and rye (secalines), and show a close relation to the taxonomy and toxic properties of wheat cereal that affect people with coeliac disease.¹⁵

Although several gluten epitopes are immunostimulatory, some are more active than others. An immunodominant peptide of 33 aminoacids (residues 57–89) identified from an α -gliadin fraction has functional properties attributable to many proline and glutamine residues.¹⁶ Proline gives the peptide increased resistance to gastrointestinal proteolysis (in people with and without coeliac disease), and causes a left-handed helical conformation, which strengthens binding with HLA-DQ2 and HLA-DQ8 molecules on antigen-presenting cells. Additionally, glutamine residues are a preferred substrate for tissue-transglutaminase-mediated deamidation, which confers an enhanced immunogenicity.

Genes

Genetic factors are a likely cause of coeliac disease on the basis of familial aggregation¹⁷ and a concordance rate of about 85% between monozygotic twins.¹⁸ Results from genetic linkage studies show that the disease is strongly associated with HLA-DQ genes. Most patients carry a variant of DQ2 (alleles DQA1*05/DQB1*02) and others carry a variant of DQ8 (alleles DQA1*03/DQB1*0302).¹⁹ Rare DQ2-/DQ8- patients carry alleles that code for one chain of the DQ2-encoded heterodimer (DQA1*05 or DQB1*02).²⁰ The association between HLA genes (COELIAC1 locus on chromosome 6p21) and coeliac disease is very strong compared with other HLA-linked diseases; however, researchers estimate that the genetic effect attributable to HLA is 53%.²¹ Moreover, DQ2 is carried by roughly a third of the general population, thus suggesting that HLA is only partly the cause of the condition. The concordance rate between HLA-identical siblings is much lower than between monozygotic twins;¹⁸ thus, other non-HLA regions must be involved.²² Furthermore, researchers report that additional susceptibility might be conferred by: COELIAC2 (5q31–33),²³ which contains cytokine gene clusters;

COELIAC3 (2q33)²⁴ that encodes the negative co-stimulatory molecule CTLA4; and COELIAC4 (19p13.1),²⁵ which contains the myosin IXB gene variant encoding an unconventional myosin that alters epithelial actin remodelling. Results of two genome-wide association studies have shown risk variants in the region harbouring interleukin 2 and interleukin 21 (4q27)²⁶—which are both implicated in intestinal inflammation—and six other genetic risk variants controlling immune responses (figure 1).²⁷ Fine mapping and deep resequencing of these regions is needed to establish their causal relation to coeliac disease.

Environmental cofactors

Some drugs can have a role in enhancing a person's susceptibility to gluten—Cammarota and colleagues²⁸ concluded that a course of interferon alfa could activate coeliac disease in predisposed people. Intestinal infections might cause a transient rise in small-bowel permeability and could lead to up-regulation and release of tissue transglutaminase that, in turn, enhances gluten immunogenicity. Rod-shaped bacteria have been identified in the intestinal epithelium in children with coeliac disease, although this colonisation could just be coincidental.²⁹ Results of a longitudinal study show that a high frequency of rotavirus infections could raise the risk of coeliac disease in genetically predisposed children.³⁰ The homology between the rotavirus-neutralising protein VP-7 and tissue transglutaminase might explain how rotavirus infection is implicated in the development of coeliac disease.³¹

Changes in infant-feeding practices might affect the rise and fall of the disease in Sweden. Results of a case-control study³² showed that the introduction of dietary gluten while infants were still being breastfed, and the introduction of small or medium amounts rather than large amounts, are independent protective factors against the disease in early and perhaps later childhood, whereas the timing of gluten introduction alone was irrelevant. However, these results were not confirmed by a subsequent prospective study.³³ Large follow-up studies are, therefore, needed to clarify how dietary cofactors affect the development of this condition before a child's immunity is established and to identify primary prevention strategies.

Pathophysiology

Study of the pathogenesis of coeliac disease has focused on the mechanisms by which gluten peptides, after crossing the epithelium into the lamina propria, are deamidated by tissue transglutaminase and then presented by DQ2+ or DQ8+ antigen-presenting cells to pathogenic CD4+ T cells. Once activated, the CD4+ T cells drive a T-helper-cell type 1 response that leads to the development of coeliac lesions—namely intraepithelial and lamina propria infiltration of inflammatory cells, crypt hyperplasia, and villous atrophy (figure 2).

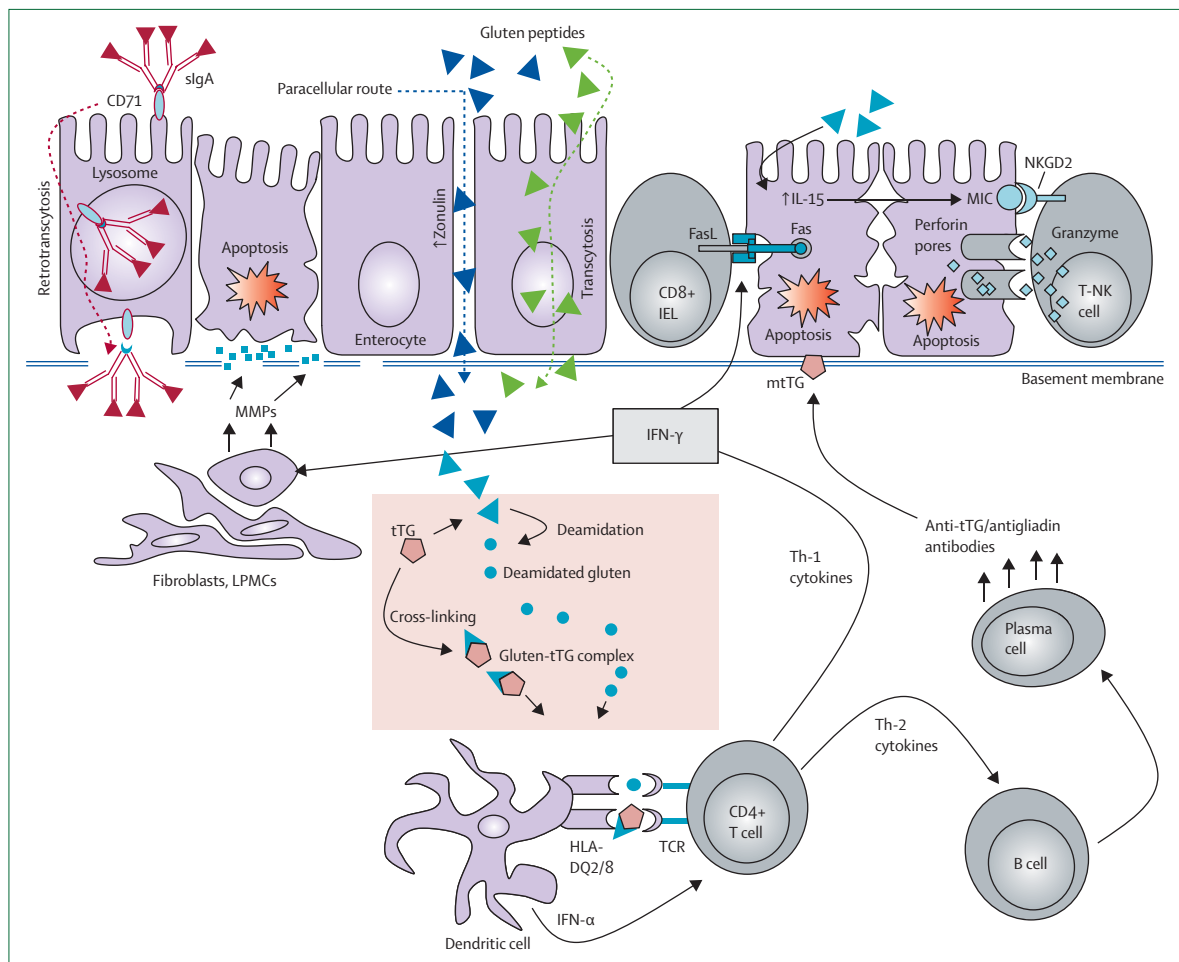


Figure 2: Mechanisms of mucosal damage in coeliac disease

Gluten peptides can be transported across the intestinal epithelium either paracellularly (blue route) as a consequence of impaired mucosal integrity attributable to increased release of zonulin, or via transcytosis (green route), or retrotranscytosis of secretory IgA (sIgA) through transferrin receptor CD71. Deamidation or crosslinking of gluten by tissue transglutaminase (tTG) (beige panel) reinforces presentation of gluten peptides by dendritic cells to CD4+ T cells in the context of HLA-DQ2 or HLA-DQ8 molecules. Activated gluten-reactive CD4+ T-cells produce high levels of pro-inflammatory cytokines, thus inducing a T-helper-cell-type-(Th)1 pattern dominated by interferon gamma (IFN- γ). Th-1 cytokines promote inflammatory effects including fibroblast or lamina propria mononuclear cell (LPMC) secretion of matrix metalloproteinases (MMPs), which are responsible for degradation of extracellular matrix and basement membrane, and increased cytotoxicity of intraepithelial lymphocytes (IELs) or natural killer (NK) T cells. These latter facilitate the apoptotic death of enterocytes by the Fas/Fas ligand (FasL) system, or interleukin 15 (IL-15)-induced perforin-granzyme and NKG2D-MIC signalling pathways, thus leading to enterocyte apoptosis. Interferon alpha (IFN- α) released by activated dendritic cells perpetuates the inflammatory reaction by inducing CD4+ T cells to produce IFN- γ . Additionally, through the production of Th-2 cytokines, activated CD4+ T-cells drive the activation and clonal expansion of B cells, which differentiate into plasma cells and produce anti gliadin and anti-tTG antibodies. By interacting with the extracellular membrane-bound tTG (mtTG), tTG-autoantibody deposits in the basement-membrane region might induce enterocyte cytoskeleton changes with actin redistribution and consequent epithelial damage.

Epithelial translocation of gluten peptides

In the intestinal lumen, gastrointestinal proteases are a first defence against potentially toxic dietary proteins, including the incompletely digested gluten proteins.¹⁶ Gut permeability is enhanced in coeliac disease and gluten can reach the lamina propria through different routes (figure 2). Investigators have postulated that there is a paracellular route on the basis of amplified expression of zonulin—a protein implicated in the opening of tight junctions³⁴—and T-helper-1-induced changes in the expression, localisation, or phosphorylation of epithelial junctional proteins in active disease.³⁵ Additionally,

Wapenaar and co-workers³⁶ identified variants in genes coding for tight junctional proteins, suggesting that heritable factors might contribute to this effect. However, the paracellular passage of gluten is not proven, whereas study results have shown that the immunodominant $\alpha 2$ -gliadin-33mer¹⁶ translocates into the lamina propria via an interferon- γ -dependent transcytosis,³⁷ which suggests involvement of the transcellular route. Furthermore, the protected transport pathway, which is driven by retrotranscytosis of secretory IgA through transferrin receptor CD71,³⁸ promotes the influx of intact, and thus harmful, gluten peptides.

Modification and presentation of gluten peptides

Tissue transglutaminase, a calcium-dependent, ubiquitous enzyme that catalyses post-translational modification of proteins and is released during inflammation, could have at least two crucial roles in coeliac disease: as the main target autoantigen for antiendomysial antibodies and hTTG antibodies,³⁹ and as a deamidating enzyme that raises the immunostimulatory effect of gluten.^{40,41} Expression and activity of tissue transglutaminase are raised in the mucosa of patients with coeliac disease,⁴² where, by deamidating glutamine to glutamic acid, this enzyme makes gliadin peptides negatively charged and therefore more capable of fitting into pockets of the DQ2/DQ8 antigen-binding groove.^{41,43} Additional functions of the enzyme in coeliac disease consist of cross-linking gluten peptides, thus forming supra-molecular complexes, and catalysing either the binding of gluten peptides to interstitial collagen⁴⁴ or the incorporation of histamine into gluten proteins (transamidation).⁴⁵ All of these actions contribute to the formation of a wide range of T-cell-stimulatory epitopes that might be implicated in different stages of the disease.⁴⁶ The α 2-gliadin-33mer fragment¹⁶ is the most immunogenic because it harbours six partly overlapping DQ2-restricted epitopes.⁴⁷

Effector mechanisms

In the mucosa of patients with active coeliac disease, gluten-reactive CD4+ T cells produce several pro-inflammatory cytokines, interferon γ being dominant,^{48,49} that trigger various effector mechanisms including raised secretion of tissue-damaging matrix metalloproteinases⁵⁰ and heightened cytotoxicity of intraepithelial lymphocytes against enterocytes with increased enterocyte apoptosis and villous flattening (figure 2).⁵¹

Several aspects of the molecular mechanisms that drive the immune response in coeliac disease are unknown. Some proinflammatory cytokines are upregulated in active coeliac mucosa—namely interferon γ ,^{48,49} interferon α ,⁵² interleukin 6,⁵³ interleukin 18,⁵⁴ and interleukin 21⁵⁵—however, paradoxically, tumour necrosis factor α ,⁵⁶ the most powerful promoter of inflammation, and interleukin 12,⁴⁸ the main cytokine that primes T cells for interferon γ production, are not raised. Di Sabatino and colleagues⁵⁷ showed the pathogenic function of interferon- α -producing plasmacytoid dendritic cells, which draws attention to the crucial role of interferon α in promoting the differentiation of type 1 T-helper-cells and production of interferon γ .

T-cell-mediated immunity alone does not account for the expansion of cytotoxic CD8+ intraepithelial lymphocytes. Interleukin 15 is implicated in activating perforin–granzyme-dependent cytotoxicity by coeliac intraepithelial lymphocytes,^{58,59} and in promoting their expression of the natural-killer receptors CD94 and NKG2D,^{60,61} thus contributing to enhanced enterocyte apoptosis.^{62,63} Furthermore, interleukin 15 might have a

Panel 1: Range of clinical presentations in coeliac disease

Silent coeliac disease

- Patients who do not complain of any symptoms and do not seek medical advice
- Most of these patients are relatives of patients with known coeliac disease or members of the general population found to be positive at the search for antiendomysial antibodies or hTTG antibodies

Minor coeliac disease

- Patients complaining of trivial, transient, or apparently unrelated symptoms (dyspepsia, abdominal discomfort and bloating, mild or occasional altered bowel habit without malabsorption mimicking irritable bowel syndrome, unexplained anaemia, isolated fatigue, cryptic hypertransaminasaemia, infertility, peripheral and central neurologic disorders, osteoporosis, short stature, dental enamel defects, dermatitis herpetiformis), or of isolated symptoms of autoimmune diseases often reported in association with coeliac disease
- Most of these patients are biopsied after positive search of antiendomysial antibodies or hTTG antibodies

Major coeliac disease

- Patients complaining of frank malabsorption symptoms (diarrhoea which is often nocturnal and with incontinence, steatorrhoea suggested by loose discoloured, greasy, and frothy stools that are difficult to flush away, weight loss and other features of malnutrition, cramps, tetany, and peripheral oedema due to electrolyte and albumin depletion); symptoms of other autoimmune diseases may be associated
- Most of these patients are biopsied only on the basis of symptoms

crucial role in the emergence of T-cell clonal proliferations because of its antiapoptotic action on the intraepithelial lymphocytes,⁵⁹ therefore predisposing patients to the malignant complications of coeliac disease.⁵⁸

Some gluten peptides can directly induce mucosal damage via a non-T-cell-dependent pathway (innate response). The best characterised peptide is the non-immunodominant p31-43/49 fragment of α -gliadin that is thought to be unable to stimulate gluten-reactive CD4+ T cells.^{61,62} In coeliac mucosa, p31-43/49 induces interleukin 15 production, which in turn inhibits the immune-regulatory signalling of transforming growth factor β ,⁶³ promotes dendritic cell maturation, and causes epithelial stress.

Autoantibodies against tissue transglutaminase might contribute to mucosal damage by preventing the generation of the active form of transforming growth factor β ,⁶⁴ or through inducing enterocyte cytoskeleton changes with actin redistribution via their interaction with the extracellular-membrane-bound tissue transglutaminase,⁶⁵ or by stabilising tissue transglutaminase in a catalytically advantageous conformation.⁶⁶ Conversely, because the enzyme autoantibodies might inhibit the activity of tissue transglutaminase,⁶⁵ they could block their pathogenic role. However, inhibition is far from complete, and the residual enzyme activity could be sufficient to exert its pathogenic role.

Some aspects of coeliac disease pathogenesis are unknown, including the relation between the events in

the epithelium, the contribution of innate or adaptive immunity, the role of regulatory T cells, and the possible function of gliadin peptides as ligands for mammalian-pattern-recognition receptors.

Clinical aspects

Mode of presentation

Until 30 years ago, the use of intestinal biopsy was reserved for patients with symptoms of overt malabsorption and, consequently, the prevalence of malabsorption among patients with coeliac disease was very high. At the beginning of the 1980s, awareness of the disease improved and a lowered threshold for its investigation led us to acknowledge the more subtle and variable clinical expression of the condition. At the end of the 1980s—after the advent of serology—the number of patients with minor symptoms was twice the number of people with overt malabsorption, and this observation was accompanied by a significant rise in the rate of diagnosis, a progressive lowering of patients' age at diagnosis, and a reduction of the female to male ratio.⁶⁷ The initial trend was seen in Italy and similar trends were confirmed in the USA.⁶⁸ The disease is two or three times more common in women than in men, but this predominance falls after the age of 65 years.⁶⁹

The clinical range of coeliac disease is very wide—patients can be asymptomatic to severely symptomatic. To categorise the possible forms of clinical presentations, terms such as atypical, typical, and classic should be discouraged, whereas the terms silent, minor, and major can characterise clinical presentation simply and clearly (panel 1). Use of the term silent has, however, been criticised because a thorough history, examination, or laboratory investigation might reveal very subtle abnormal changes,⁷⁰ although many patients with silent disease are symptom-free.

Autoimmune and immune-mediated diseases often reported in association with coeliac disease are type 1 diabetes, autoimmune thyroiditis, autoimmune myocarditis, idiopathic dilated cardiomyopathy, Sjögren's syndrome, systemic lupus erythematosus, autoimmune hepatitis, autoimmune cholangitis, primary biliary cirrhosis, IgA deficiency, Addison's disease, IgA mesangial nephropathy, alopecia areata, neurological abnormalities, atopy, inflammatory bowel disease, systemic and cutaneous vasculitis, psoriasis, juvenile idiopathic arthritis, and polymyositis.

The mechanisms that cause the severity of clinical presentation remain unknown. Researchers have showed that neither the degree of duodenal villous atrophy⁷¹ nor the extent of visible enteropathy assessed by capsule endoscopy⁷² correlates with presentation. These results should be corroborated by accurate tests such as morphometry to quantify mucosal damage and multiple biopsy collection and analysis to assess the length of small intestine affected.

Comorbidity

Some patients with coeliac disease are affected by a loss of bone mass grossly proportional to the degree of malabsorption.^{73,74} In children, osteoporosis can be reversed by a gluten-free diet with restoration of normal peak densitometric values,⁷⁵ however, in adults, bone-mineral density improves but rarely normalises.⁷⁴ Accordingly, a higher risk of fractures has been reported in adults with coeliac disease.^{76,77}

Screening bone-mineral density at diagnosis seems not to be justified in view of a study⁷⁸ showing a very low prevalence of osteoporosis in an unselected cohort of untreated patients with coeliac disease attending a district general hospital. Densitometry might be restricted to patients with major coeliac disease and particularly to those with previous pathological fractures, untreated hypogonadism, concomitant lactose intolerance, poor compliance with or unresponsiveness to a gluten-free diet, or those on steroids. Most bone-mineral density improvement occurs after one or two years of gluten withdrawal,⁷⁴ thus, the optimal time of measurement might be at this time when densitometry is indicated to evaluate the need to supplement the gluten-free diet with mineral active drugs.

Autoimmune disorders occur much more frequently in patients with coeliac disease than in the general population. The prevalence of associated autoimmunity was shown to increase substantially with increasing age at diagnosis—which is taken as an index of the duration of exposure to gluten—in a cohort of coeliac children and adolescents.⁷⁹ However, these results were not confirmed in adult patients when mean duration of actual gluten exposure was considered rather than age at diagnosis.⁸⁰ Protection against autoimmune associations has been held as one of the strongest arguments in favour of a prompt diagnosis and treatment, this issue should be carefully reconsidered.

The clinical relevance of autoimmunity in coeliac disease is threefold: first, it further deteriorates the clinical course of coeliac disease; second, patients might present only with symptoms of secondary autoimmunity and this could favour the diagnosis of minor coeliac disease; and third, gluten withdrawal could improve the control of some associated autoimmune disorders.

Mild liver abnormalities are common in coeliac disease. Isolated hypertransaminasaemia, without specific histological changes and promptly reversible with a gluten-free diet, is the most common form of hepatic involvement. Other possible forms include autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and non-alcoholic fatty liver disease.⁸¹

A wide range of neurological and psychiatric disorders such as ataxia, peripheral neuropathy, epilepsy (mainly with occipital calcifications), headache, dementia, depression, autism, and schizophrenia has been reported in association with coeliac disease.⁸² However, pathology studies and diet trials have not confirmed the existence of

Panel 2: Risk groups for coeliac disease case-finding

- First-degree relatives
- Dermatitis herpetiformis
- Unexplained iron deficiency anaemia
- Autoimmune thyroiditis
- Type 1 diabetes
- Unexplained infertility
- Recurrent abortion
- Dental enamel hypoplasia
- Cryptic hypertransaminasaemia
- Autoimmune liver disease
- Short stature
- Delayed puberty
- Down's syndrome and Turner syndrome
- Irritable bowel syndrome
- Unexplained osteoporosis
- Sjögren's syndrome
- Epilepsy with occipital calcifications

a link between autism and schizophrenia and coeliac disease. Furthermore, women with coeliac disease more frequently experience recurrent spontaneous miscarriage, delayed menarche, early menopause, and amenorrhoea. Fertility problems and loss of libido can also be present in male patients.⁸³

Natural course

Our understanding of the natural course of coeliac disease is very limited. At diagnosis, we have difficulty dating the development of intestinal lesions and symptoms (if any) and predicting what the spontaneous course of the disease would be without a gluten-free diet. We do know gluten might not be toxic from the first contact. Children and adults with normal results on intestinal biopsy while taking a gluten-containing diet can subsequently develop villous atrophy that recovers after commencing a gluten-free diet.⁸⁴ The recognition of a precoeliac state is usually retrospective and this condition has been termed

latent coeliac disease.⁸⁵ Patients with positive coeliac-related antibodies but with normal mucosa are regarded as having potential coeliac disease,⁸⁶ although there is no evidence to support managing these patients with a gluten-free diet or careful follow-up until unequivocal mucosal flattening is recorded.

Mortimer and colleagues reported that adult patients with coeliac disease, diagnosed in childhood and then lost to follow-up, had mild to moderate clinical complaints and persistence of flat mucosa after many years of gluten exposure.⁸⁷ Matysiak-Budnik and co-workers,⁸⁸ who undertook a similar study in patients who resumed a gluten-containing diet, reported most patients had some villous atrophy. However, in 20% of patients duodenal biopsy remained normal, indicating an evolution towards latency that might be only transient, as suggested by a subsequent histological relapse in two patients and by the high frequency of coeliac antibodies in this subgroup. These results, therefore, do not disprove that coeliac disease is a permanent condition.

Diagnosis**Case finding**

The burden of undetected disease is very high: a cautious prevalence estimate of 0.5% equates to around 2.5 million patients still undiagnosed in Europe, and researchers have calculated that the ratio between diagnosed and undiagnosed patients is as high as 1:7.⁸⁹ Increased physician awareness of the clinical range of this disorder and a continued high threshold of suspicion are, therefore, needed. Researchers have shown that case-finding by measurement of antiendomysial antibodies or hTTG antibodies followed by histological confirmation on duodenal biopsy is an accurate, cost-effective, and acceptable approach for diagnosis—even in primary care.^{90,91} Additionally, patients with conditions that are frequently associated with coeliac disease should be considered for screening (panel 2).⁹² This case-finding strategy is preferred to mass screening of the general

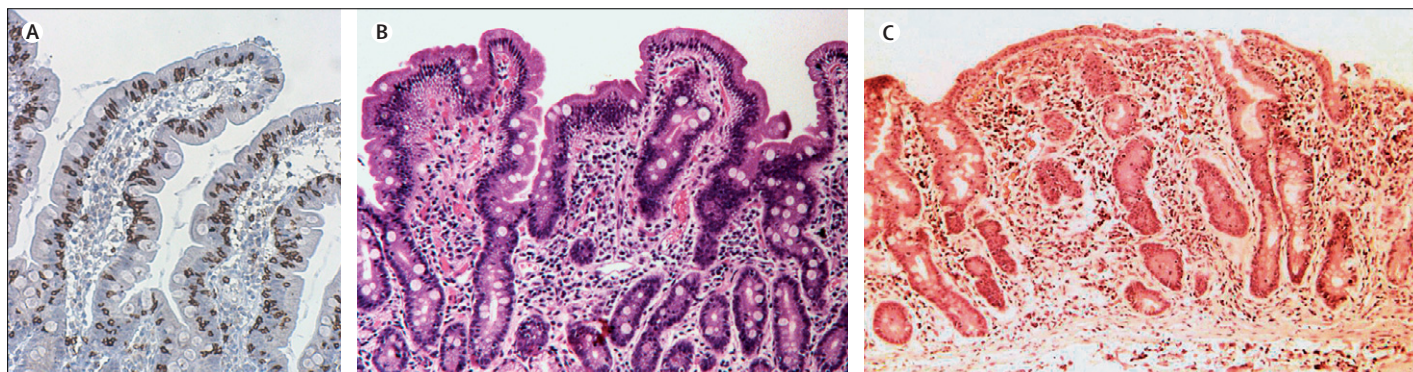


Figure 3: Grades A, B1, and B2 coeliac disease

Representative pictures of coeliac disease grades. (A) Grade A (infiltrative non-atrophic lesions, haematoxylin and eosin stain [H&E], and CD3 staining). (B) Grade B1 (atrophic with shortened but still detectable villi). (C) Grade B2 (atrophic with no longer detectable villi, H&E staining).⁹⁵

Panel 3: Other causes of flat mucosa

- Autoimmune enteropathy
- Tropical sprue
- Giardiasis
- HIV enteropathy
- Bacterial overgrowth
- Crohn's disease
- Eosinophilic gastroenteritis
- Cow's milk enteropathy
- Soy protein enteropathy
- Primary immunodeficiency
- Graft-versus-host disease
- Chemotherapy and radiation damage
- Protein energy malnutrition

population, although coeliac disease meets the criteria for mass screening for at least four reasons: the disease is common, there are simple and sufficiently accurate screening tests, it has an agreed treatment, and it is burdened by the risk of complications. However, effectiveness, acceptability, and the costs of mass screening are unknown and there is no evidence that this alternative strategy would lead to reduced morbidity and mortality.

Histology

The diagnosis of coeliac disease is based on the presence of characteristic lesions in small-intestinal biopsy samples. Four endoscopic biopsies are mandatory for absolute diagnostic confidence,⁹³ and the specimens should be correctly oriented and sectioned to avoid artifacts that frequently cause misdiagnosis. Biopsy samples are usually taken from the distal duodenum; however, isolated patchy villi atrophy of the duodenal bulb has been reported,⁹⁴ so an additional biopsy from this region should also be taken.

The pathology of the disease can range from infiltrative lesions characterised by increased intraepithelial lymphocyte with normal architecture to completely flat mucosa. The Marsh classification, as modified by Oberhuber and colleagues,⁹⁵ is used to grade the severity of these lesions. However, this classification entails six diagnostic categories and can be unreliable. A simplified grading system based on three morphologies has been proposed by Corazza and colleagues⁹⁶ (figure 3): A, infiltrative non-atrophic lesions; B1, atrophic lesions with shortened but still detectable villi; and B2, atrophic lesions with undetectable villi. This system has an inter-observer reproducibility that is significantly higher than is the Marsh-Oberhuber classification. Minor lesions, such as increased intraepithelial lymphocytes, are recognised to be important for diagnosis; however, isolated infiltrative lesions have a low specificity for coeliac disease and other causes of intraepithelial lymphocytosis have been recently reviewed.⁹⁷ Moreover, villi atrophy is reported in other disorders (panel 3), and diagnosis of coeliac disease is,

therefore, confirmed only by clinical symptoms, a positive serology, or histological improvement after commencement of a gluten-free diet. Assessment of clinical improvement in patients with silent or minor coeliac disease is very difficult and these patients could benefit from a second post-treatment biopsy. A repeat biopsy after dietary intervention is not always necessary, but showing of histological improvement supports the diagnosis, checks dietary compliance, and reassures the patient. Furthermore, a second biopsy can be useful for patients with ambiguous histological changes, initial negative or discrepant serology, or continued symptoms.⁹⁸

Serology

Hill and colleagues⁹⁹ reviewed studies assessing the accuracy of antibody tests. In 32 studies of antiendomysial antibody IgA, sensitivity ranged from 86% to 100% (mean 95%) and specificity from 90% to 100% (mean 99%); in 27 studies for tissue transglutaminase IgA, sensitivity ranged from 61% to 100% (mean 87%) and specificity from 86% to 100% (mean 95%). These studies should, however, be interpreted with caution: all were done in research settings where accuracy is probably higher than in clinical practice, most assessed preselected serum samples and not prospectively enrolled individuals, and the use of guinea-pig enzyme instead of human-recombinant enzyme in some studies might have lowered the specificity of the tissue transglutaminase results. Researchers of a prospective study that compared antiendomysial IgA with hTTG IgA reported a similar optimum sensitivity but a significantly lower specificity for the hTTG IgA.¹⁰⁰ Antiactin antibodies, which correlate with intestinal damage, do not have a role in coeliac disease screening,¹⁰¹ whereas antibodies against deamidated gliadin peptides reinforce the accuracy of and perform better than hTTG antibodies and antiendomysial antibodies in the assessment of dietary compliance and post-treatment mucosal recovery.¹⁰² A potential pitfall of serology is the 10–16 fold increase of IgA deficiency in the disease. Serum IgA should, therefore, always be checked and, if absent, measurement of antiendomysial IgG and hTTG IgG is recommended.¹⁰³ Measurement of total IgA and antiendomysial IgA or hTTG IgA—depending on local facilities and laboratory expertise—is a suitable approach to screen for coeliac disease. However, although serology is a good method to select patients for biopsy, negative serology should not preclude biopsy examination in individuals for whom disease is suspected on clinical grounds.

Endoscopy

Two major endoscopic abnormal changes—the disappearance or reduction of Kerkring folds¹⁰⁴ and the scalloped configuration of reduced folds (figure 4)¹⁰⁵—were identified together. Conceivably, in these early studies, researchers overestimated the performance of endoscopic markers because of the high pretest probability of coeliac

disease, and subsequent reports have downsized their sensitivity and specificity.^{106,107} Controversies could be solved by defining the appropriate setting for endoscopy in coeliac disease. Endoscopic markers have no role in diagnosis when disease is suspected on clinical or serological grounds. In these patients, the decision to do biopsies should not depend on endoscopy. However, the recognition of abnormal changes on endoscopy done for other reasons might be crucial for patients in whom coeliac disease is not suspected. Immersion endoscopy has been proposed by Cammarota and colleagues¹⁰⁸ as a cost-sparing and very accurate biopsy-avoiding technique, although it is unable to detect infiltrative lesions and its use is not feasible in a routine endoscopy setting.

HLA typing

The negative predictive value of HLA-DQ2/DQ8 is almost absolute and is useful for ruling out coeliac disease in high-risk individuals such as first-degree relatives and patients with type 1 diabetes. Negative results avoid future concerns about the condition and reduce the cost of further serological tests; the limiting factor is the high frequency of these predisposing genes in many of the risk groups susceptible to case-finding.¹⁰⁹

Dietary treatment

The only proven treatment for coeliac disease is strict and life-long adherence to a gluten-free diet. All food and drugs that contain gluten from wheat, rye, barley, and their derivatives must be eliminated because even small amounts can be harmful. Gluten contamination in gluten-free products cannot be completely avoided; results of a double-blind placebo-control trial established that 10 mg gluten per day is tolerated whereas 50 mg is harmful.¹¹⁰ Individual variability between patients, however, makes setting a universal safe threshold difficult.

Apart from the ubiquity of gluten in foodstuffs, another crucial limit of a gluten-free diet is patients' compliance, which is imperfect—particularly in teenagers,¹¹¹ adults,¹¹² and patients diagnosed in screening programmes.¹¹³ The main factors associated with poor compliance are those that substantially decrease quality of life.¹¹⁴ Anxiety, constipation and intestinal bloating, changes in body composition and dietary intake, and poor vitamin status are additional minor but well defined side-effects associated with this lifelong treatment.

Although coeliac antibodies usually disappear after commencement of a gluten-free diet, they are not accurate for detecting dietary lapses¹¹⁵ or mucosal recovery.¹¹⁶ Antiendomysial antibodies are a more precise measure than are hTTG antibodies,¹¹⁵ and antibodies against deamidated gliadin might be effective.¹⁰² Dietary compliance assessed by a trained interviewer is considered the best measure of coeliac disease control

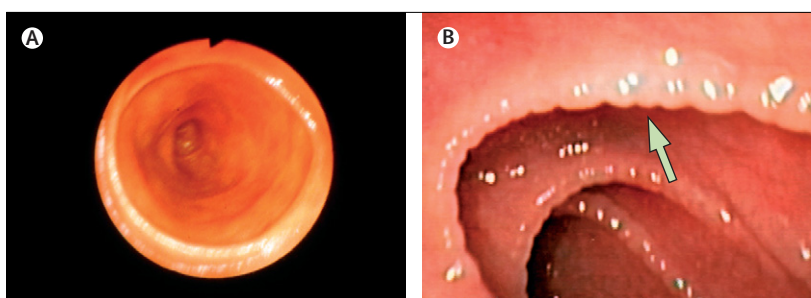


Figure 4: Kerkring folds and scalloped configuration

Endoscopic view of markedly reduced (A) and scalloped configuration of folds (arrow) in active coeliac disease (B).

because it is low cost, non-invasive, and correlates to intestinal damage.¹¹⁷

Oats seem to be safe in coeliac disease, even after 5 years of exposure,¹¹⁸ but their inclusion in a gluten-free diet is restricted by reports of oat-sensitive patients.^{119,120} Avenin-reactive mucosal T cells have been identified in these patients, and avenin peptides have sequences rich in proline and glutamine residues that closely resemble wheat-gluten epitopes.¹²⁰ Patients' education, close supervision with regular nutritional counselling, and maintenance of dietary adherence when travelling or dining out are crucial for achieving compliance.⁶⁹ Patients should be encouraged to join a coeliac-disease support group because members are usually more knowledgeable and adherent to their diet than are non-members.

Complications

Results of studies from Italy¹²¹ and Sweden¹²² showed a twofold increased mortality in patients with coeliac disease, whereas this risk was only marginally raised in the UK.¹²³ The risk of death rose with increasing delay in diagnosis and in patients with poor dietary compliance; non-Hodgkin lymphoma was the main cause of death.¹²¹ Patients diagnosed with the condition in childhood have a threefold increased risk of long-term mortality.¹²⁴ However, this excess was mostly attributable to external causes as a possible result of behavioural changes

	Type 1	Type 2
Unresponsive villous atrophy	Yes	Yes
Associated autoimmune diseases	Yes	No
Aberrant T-cell phenotype	≤10% of IELs	>50% of IELs
Clonal TCR-γ gene rearrangement	+	++
Chromosomal abnormalities	No	Yes
HLA-DQ2 homozygosity	Uncommon	Common
Associated ulcerative jejunoileitis	Rare	Common
Response to immunosuppressants (steroids, budesonide, azathioprine, tacrolimus, infliximab)	Yes	No
Risk of developing overt EATL	Low	37–60% within 5 yrs
Mortality rate	Slightly increased	5-yr survival <50%

EATL=enteropathy-associated T-cell lymphoma. IEL=intraepithelial lymphocyte. TCR=T-cell receptor.

Table 1: Comparison between refractory coeliac disease type 1 and type 2

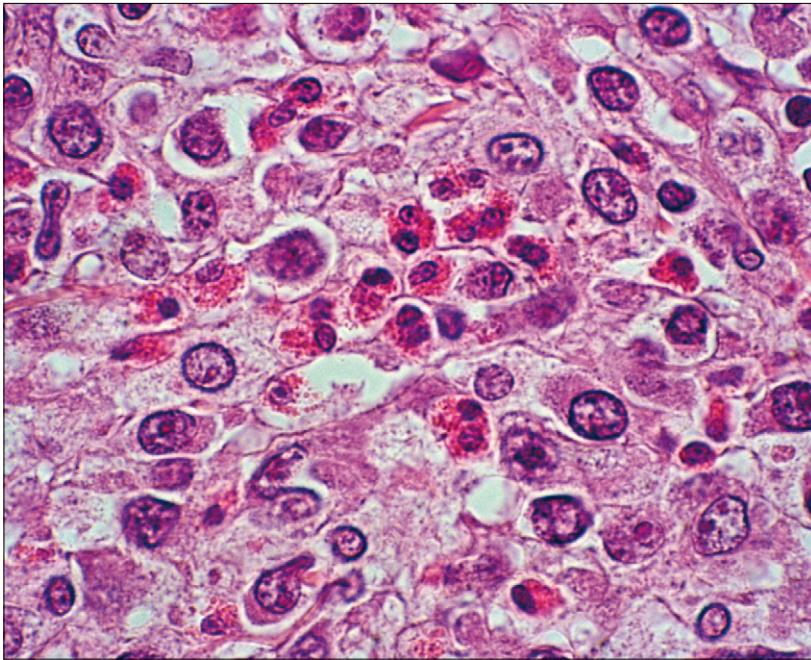


Figure 5: Features of enteropathy-associated T-cell lymphoma

Medium-sized to large tumour cells with round or angulated vesicular nuclei, prominent nucleoli, and moderate to abundant, pale-staining cytoplasm. A heavy eosinophil infiltrate is evident between the tumour cells. Haematoxylin and eosin stain.

associated with this chronic disease and its treatment. No increased risk of cancer or death has been reported in patients with dermatitis herpetiformis.¹²⁵

Adult patients can develop complications such as refractory coeliac disease, ulcerative jejunoileitis, and enteropathy-associated T-cell lymphoma through a progressive accumulation in the intestinal epithelium of aberrant (CD3ε+, CD103+, CD8-, CD4-, T-cell-antigen receptor αβ-) and clonal (restricted rearrangements of T-cell-antigen receptor γ chain) intraepithelial lympho-

cytes,¹²⁶ abnormally expanded by interleukin 15.^{58,59} These diseases are a continuum of each other, in which phenotypic disturbances of intraepithelial lymphocytes and chromosomal rearrangements^{127,128} cause the self-perpetuating, gluten-independent tissue damage of refractory coeliac disease and the subsequent uncontrolled expansion of a neoplastic T-cell clone of enteropathy-associated T-cell lymphoma.¹²⁹

Refractory disease is defined by the absence of histological, and thus clinical, response to a gluten-free diet. Refractoriness is very often only apparent and mostly attributable to, dietary non-compliance or unintentional gluten intake. Other reasons for apparent refractoriness include misinterpretation of the original biopsy, slow histological recovery after diet, and misdiagnosis with other causes of villi atrophy (panel 3). Among them, autoimmune enteropathy may be easily differentiated by the presence of anti-enterocyte autoantibodies.¹³⁰ Refractory coeliac disease has no relation to the persistence, after villi regrowth, of diarrhoea, which could be caused by concomitant irritable bowel syndrome, pancreatic insufficiency, microscopic colitis, and lactose malabsorption.¹³¹ Refractory coeliac disease could affect about 5% of patients with the condition,^{132,133} although this figure, which is derived from tertiary referral centres, might not reflect true prevalence. There are two categories of true refractory disease—type 1 and type 2 (table 1).^{132–134}

Ulcerative jejunoileitis, which shares many immunopathological features with type 2 refractory coeliac disease,¹³⁵ is characterised by multiple ulcerations that evolve in strictures of the intestinal wall. Colicky central abdominal pain, distension, low-grade fever, diarrhoea, and weight loss are the main clinical manifestations. The rate of death is very high—resulting from obstruction, bleeding, and perforation.

Enteropathy-associated T-cell lymphoma is mainly localised in the proximal small intestine, is more prevalent in men over 60 years, and patients have a very poor outcome with a 2-year survival rate of 15–20%.¹³³ Gross examination of the bowel reveals multifocal ulcerating nodules that can be accompanied by strictures and perforation (see figure 5 for histological features). Clinicians should be alerted to this complication by unexplained weight loss, abdominal pain, diarrhoea, loss of albumin and blood, increased lactate dehydrogenase, fever, and night sweating.¹³⁶ Clinical suspicion should lead to an extensive diagnostic work-up (table 2); ¹⁸F-FDG-PET and histological identification of lesions are regarded as the best options for diagnosis.

Refractory type 2 coeliac disease is resistant to available treatments, and although new drugs are being used, no randomised studies have been undertaken. These drugs include the antiCD52 antibody alemtuzumab,¹³⁷ cladribine,¹³⁸ and high-dose chemotherapy given before autologous haemopoietic stem-cell transplantation.¹³⁹ The latter produced encouraging results in early-stage

	Pros	Cons
Small bowel enema	Visualisation of the entire small bowel	Only moderate accuracy
Abdominal computed tomography	Visualisation of extraintestinal findings (lymphadenopathy, spleen atrophy)	No specific pattern identified
PEG-enhanced magnetic resonance	Visualisation of extraintestinal findings	Accuracy not tested in complicated coeliac disease
¹⁸ F-FDG-positron emission tomography	High sensitivity and adequate specificity; possibly helpful in follow-up studies	Only preliminary prospective data; active peristalsis and inflammation may affect specificity
Capsule endoscopy	Direct visualisation and magnification of the entire small bowel; well tolerated; best sensitivity for ulcerations	Capsule retention in the event of strictures; unfeasibility for collecting biopsies
Double balloon enteroscopy	Feasibility for collecting biopsies; allows a definitive diagnosis	Only partial small bowel visualisation
Explorative laparotomy	Allows full thickness biopsies and operative procedures	Invasive

PEG=polyethylene glycol. FDG=fluoro-deoxy-glucose.

Table 2: Diagnostic options in enteropathy-type T-cell lymphoma

Target	Therapeutic agent	Mechanism of action	
Gluten peptides	Prolyl endopeptidases (PEP)	Cleavage of proline-rich and glutamine-rich gliadin peptides in safer sequences	
Zonulin	Zonulin receptor antagonist (AT-1001)	Prevention of epithelial translocation of gluten peptides into the lamina propria	
Interleukin 15	Anti-interleukin 15 antibody (AMG714)	Reduced cytolytic activity of intraepithelial lymphocytes against epithelial cells with consequent decrease of enterocyte apoptosis	
Tissue transglutaminase	Tissue transglutaminase inhibitors	Blockade of deamidation and subsequent immunological potentiation of gluten peptides	
HLA-DQ2/DQ8 molecules	DQ2/DQ8 inhibitors	Blockade of presentation of gluten peptides with consequent silencing of gluten-reactive T cells	
Dendritic cells	Peptide vaccines	Manipulation of dendritic cells in order to make them a vehicle for peptide vaccines	
Interferon γ	Anti-interferon γ antibody (fontolizumab)	Down-regulation of the Th1-mediated inflammatory response	
T cells	Anti CD3 antibody (visilizumab), anti CD4 antibody (cM-T412), anti CD25 antibody (daclizumab)	Silencing of gluten-reactive T cells	
Type 1 regulatory T cells (Tr)	Human recombinant interleukin 10 (Tenovil)	The interleukin-10-mediated expansion of type 1 regulatory T cells may suppress the immune response to gliadin	
Adhesion molecules	Anti-integrin α_4 antibody (natalizumab); anti-integrin $\alpha_4\beta_7$ antibody (MLN-02); integrin α_4 antagonist (T-0047)	Blocking the cognate interaction between integrin α_4/β_7 , expressed on lymphocytes and MAdCAM-1 expressed on mucosal endothelial cells may decrease lymphocyte recruitment in the gut	

Figure 6: Novel treatments for coeliac disease

enteropathy-associated T-cell lymphoma¹⁴⁰ but not in patients with advanced or relapsed disease.¹⁴¹ Furthermore, the efficacy of an anti-interleukin 15 monoclonal antibody aimed at reinducing the apoptotic machinery in aberrant intraepithelial lymphocytes is being investigated.^{58,59}

A third of patients with untreated coeliac disease have haematological features of splenic hypofunction,¹⁴² which can be accompanied by spleen atrophy and depletion of IgM memory-B cells that protect against encapsulated bacteria.¹⁴³ Although splenic hypofunction or atrophy is associated with a poor prognosis, its clinical relevance in coeliac disease has been overlooked—it is associated with increased autoimmunity¹⁴⁴ and pneumococcal septicaemia in adult patients.^{145,146} Thus, patients with blood-film features of splenic hypofunction—Howell Jolly bodies or red cells with membrane excavations—should receive pneumococcal-conjugate vaccine.

Future directions

Improved understanding of the molecular basis of coeliac disease has enabled researchers to suggest alternatives to a gluten-free diet. These novel treatments are aimed at blunting the immune stimulatory effects

of gluten (figure 6). However, we emphasise that some of these drugs (tissue-transglutaminase-inhibitors and monoclonal antibodies) have a poor safety profile, and their hypothetical use could, thus, be reserved for complicated forms of the disease.

Alternative approaches to manage the condition aim to eliminate detrimental gluten peptides through genetic detoxification or enzyme modification. The development of grains that have low or no immunotoxicity can be achieved with transgenic technology that deletes or silences harmful gluten sequences¹⁴⁷ or selectively breeding non-toxic wheat varieties.^{148,149} However, although the availability of non-toxic bread might resolve problems such as poor palatability and the high cost of gluten-free foods, patients' social problems would nonetheless remain.

Gluten can be detoxified within the intestine by oral administration of prolyl endopeptidases—enzymes that cleave the proline-rich and glutamine-rich immunostimulatory gluten peptides into small sequences with reduced toxic effects. In a pilot clinical study,¹⁵⁰ patients with remitting coeliac disease were given a prolyl endopeptidase from *Flavobacterium meningosepticum* and challenged with oral gluten. The results were inconclusive: fat and xylose malabsorption, instead of

histology, was used to measure the efficacy of the enzyme supplement, which did not resolve the malabsorption in about half of the patients. Additional management strategies could include the combination of two gastrically active enzymes (eg, a glutamine-specific endoprotease from barley and a prolyl endopeptidase from *Sphingomonas capsulata* are effective in detoxifying immunogenic peptides both in vitro and in animals);¹⁵¹ the use of a proline-endopeptidase from *Aspergillus niger*, which accelerates gluten degradation in a dynamic system that mimics in-vivo digestion;¹⁵² the addition to sourdough of lactobacilli proteolytic against proline-rich and glutamine-rich gluten peptides,¹⁵³ or the pretreatment of flour with a *Streptomyces mobaraensis*-derived tissue transglutaminase that stops the immunostimulatory effects of flour.¹⁵⁴ These approaches are limited by the need for complete digestion of the toxic epitopes of gliadin, which has never been shown. These strategies should be investigated in large long-term clinical studies to assess safety and clinical effectiveness. The development of gluten-sensitive rhesus macaques could be a promising model of coeliac disease for testing these new treatments.¹⁵⁵

Conflicts of interest

We declare that we have no conflicts of interest.

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