

## Seminar

## Coeliac disease

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**Coeliac disease is a genetically-determined chronic inflammatory intestinal disease induced by an environmental precipitant, gluten. Patients with the disease might have mainly non-gastrointestinal symptoms, and as a result patients present to various medical practitioners. Epidemiological studies have shown that coeliac disease is very common and affects about one in 250 people. The disease is associated with an increased rate of osteoporosis, infertility, autoimmune diseases, and malignant disease, especially lymphomas. The mechanism of the intestinal immune-mediated response is not completely clear, but involves an HLA-DQ2 or HLA-DQ8 restricted T-cell immune reaction in the lamina propria as well as an immune reaction in the intestinal epithelium. An important component of the disease is the intraepithelial lymphocyte that might become clonally expanded in refractory sprue and enteropathy-associated T-cell lymphoma. Study of the mechanism of the immune response in coeliac disease could provide insight into the mechanism of inflammatory and autoimmune responses and lead to innovations in treatment.**

Coeliac disease, originally thought to occur only rarely in childhood, is now recognised as a common condition that could be diagnosed at any age. The wide range of its clinical manifestations results in patients presenting to many different specialists: gastroenterologists, endocrinologists, rheumatologists, haematologists, cardiologists, neurologists, as well as paediatricians, dermatologists, and dentists.

Characteristics include a close linkage to specific HLA alleles (DQ2 and DQ8) and precipitation of the disease by an environmental factor, gluten—ie, the storage proteins of wheat. The alcohol-soluble fraction, gliadin, has been most studied, but most or all gluten proteins are likely to be toxic in coeliac disease, as are similar proteins in barley (hordeins) and rye (secalins).<sup>1</sup> In a dose-dependent response, these proteins induce an inflammatory process in the intestine and withdrawal of them results in regression of the process.<sup>2</sup>

Because the major genetic and environmental contributors to coeliac disease are known, a unique opportunity exists to study the pathogenic mechanisms that lead to inflammatory and autoimmune disorders.<sup>3</sup>

### Classification

The clinical classification of coeliac disease is based on the presence of gastrointestinal symptoms. Symptomatic, active, or classic coeliac disease refers to presentations with diarrhoea, with or without malabsorption; whereas in asymptomatic or silent coeliac disease, gastrointestinal symptoms are absent or not prominent even though the patient might report other non-intestinal symptoms. Latent coeliac disease is thought to be present in a person who will develop coeliac disease in the future, or who has had the disease in the past, but who at the time of investigation has normal mucosa while ingesting gluten.

### Clinical significance

Symptomatic coeliac disease is associated with substantial morbidity caused by chronic gastrointestinal symptoms, weight loss, metabolic bone disease, anaemia, and general

weakness. Implications of the disease in patients with silent coeliac disease is less clear. However, most patients with silent coeliac disease do have occult manifestations, including reduced bone density, iron or folate deficiencies,<sup>4,5</sup> and associated autoimmune diseases that are often of greater clinical significance than coeliac disease. Silent coeliac disease, however, can contribute to the morbidity of chronic diseases as was shown in patients with severe liver disease of any cause for whom diagnosis of coeliac disease and treatment with a gluten-free diet resulted in improved liver function and obviated the need for liver transplantation.<sup>6</sup>

The mortality rate in patients with coeliac disease exceeds that in the general population by a factor of 1.9–3.8,<sup>7–9</sup> which is mainly due to malignant disease. The reduction in excess mortality after 1–5 years on a gluten-free diet suggests that such a diet is protective against malignant disease in patients with coeliac disease. This finding is lent support by results from a Finnish study that showed that patients on a strict gluten-free diet had no greater frequency of death than those of the general population.<sup>10</sup>

### Genetics

Coeliac disease is a multigenic disorder associated with HLA-DQ2 (DQA1\*05/DQB1\*02) or HLA-DQ8 (DQA1\*0301/DQB1\*0302). HLA-DQ2 is expressed in more than 90% of people with coeliac disease. The expression of these HLA-DQ2 or HLA-DQ8 molecules is necessary, but not sufficient, to develop the disease. Results of studies in siblings (sib recurrence risk for coeliac disease is 10%)<sup>11</sup> and identical twins (70% concordance)<sup>12</sup> suggest that HLA genes are not the main causative factor in coeliac disease. The non-HLA chromosome region most consistently linked with coeliac disease is 5q31–33,<sup>13,14</sup> located on the long arm of chromosome 5. Another potentially interesting region for a susceptibility factor is 11q.<sup>15</sup>

### Epidemiology

On the basis of clinical frequency, coeliac disease was previously regarded as rare, occurring in 1 in 3345 people worldwide.<sup>16</sup> However, serologic screening studies have

*Lancet* 2003; **362**: 383–91

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### Search strategy and selection criteria

We did a comprehensive MEDLINE search with the MeSH terms celiac disease and coeliac disease for articles published in English between January, 1997, and June, 2002. We also scrutinised reference lists of review articles on coeliac disease for extra articles.

shown the worldwide prevalence to be 1 in 266.<sup>16</sup> Such a rate establishes coeliac disease as one of the most common genetically based diseases. Similar prevalence has been reported in most European countries, South America, and the USA.<sup>17-19</sup> Reports from North Africa,<sup>20</sup> Iran,<sup>21</sup> and India<sup>22</sup> indicate the widespread occurrence of coeliac disease.

Apart from gluten, the interaction of environmental factors in coeliac disease is poorly understood. Breastfeeding and the timing of the commencement of gluten ingestion,<sup>23</sup> viral infections that promote the secretion of interferon  $\alpha$ ,<sup>24</sup> and smoking<sup>25</sup> are some of the factors that might contribute to disease occurrence.

### Clinical presentation

Coeliac disease is a disorder of the proximal small intestine that can involve the entire small intestine in some individuals. This proximal location in the small intestine often results in malabsorption of iron, folic acid, calcium, and fat-soluble vitamins with resultant iron deficiency, folate deficiency, and reduced bone density. Diarrhoea, the hallmark of symptomatic coeliac disease, is mostly due to the progression of the disease into the distal small bowel.<sup>26</sup> When only the proximal intestine is involved, patients will usually not complain of diarrhoea because the distal small intestine can compensate and absorb the products of fat and carbohydrate digestion. The onset of diarrhoea in adults with coeliac disease can be gradual or dramatic. Pregnancy, traveller's diarrhoea, gastroenteritis, or gastrointestinal surgery can act as a trigger for the development of symptoms.

Infants and young children present with diarrhoea, abdominal distention, and failure to thrive. However, vomiting, irritability, anorexia, and constipation are also common. Older children often present with extra-intestinal manifestations, such as short stature, neurological symptoms, or anaemia. There was no increased mortality in patients who received a diagnosis in childhood;<sup>7</sup> however, results of a recent survey suggest that malignant disease in childhood coeliac disease might be under-reported.<sup>27</sup>

Despite reports in the 1970s and 1980s of patients with non-diarrhoeal symptoms,<sup>28,29</sup> the diagnosis of coeliac disease is not usually considered unless diarrhoea is present. As a result, a patient with diarrhoea has remained the most common type of presentation.<sup>30</sup> Patients have a long duration of symptoms (mean of 11 years)<sup>30</sup> and there is often a delay in diagnosis. This delay is thought to be because of physicians failing to diagnose the disease rather than patients not seeking medical care.<sup>31</sup> A contributing

factor to the delay is that patients receive an alternative diagnosis such as irritable bowel syndrome.<sup>32</sup>

The availability of serological testing and active case-seeking has changed the mode of presentation of coeliac disease.<sup>33</sup> Figure 1 shows the reasons for presentation of 170 people with coeliac disease proven by biopsy, who were diagnosed since the introduction of serological testing between 1993 and 2000.<sup>34</sup> The most common symptom on first presentation was diarrhoea, although fewer than half of patients reported it. The second largest group was those diagnosed after the screening of first-degree relatives of affected individuals.

Another way that coeliac disease is identified is through the recognition of signs of villous atrophy in patients undergoing endoscopy for symptoms not typically associated with coeliac disease—eg, those associated with oesophageal reflux.<sup>4</sup> Such symptoms often improve after the start of a gluten-free diet. Endoscopic signs of villous atrophy include a reduction in the number of circular folds in the descending duodenum, scalloping of folds, mucosal fissures, and a mosaic or nodular appearance to the mucosa.<sup>35</sup> Although these endoscopic abnormalities of the duodenal mucosa are not specific to coeliac disease and are seen in other conditions such as tropical sprue, HIV enteropathy, and opportunistic infections, they are a sensitive marker of duodenal pathology.<sup>36</sup> Coeliac disease is also often diagnosed after patients seek assessment of iron concentrations and bone density.

The disease might be diagnosed because of non-gastrointestinal manifestations (panel 1). These include dermatitis herpetiformis,<sup>37</sup> an intensely pruritic vesicular rash anywhere on the body, but especially on the extensor surfaces (knees and elbows) and the scalp. Almost all patients with dermatitis herpetiformis have coeliac disease. Although dapsone controls skin lesions of dermatitis herpetiformis, a gluten-free diet allows patients to discontinue the drug, promotes healing of the intestine, and reduces the risk of lymphoma.<sup>38</sup>

Patients might present with neurological symptoms such as peripheral neuropathy,<sup>39</sup> ataxia,<sup>40</sup> or epilepsy, frequently with occipital calcifications.<sup>41</sup> Other less common presentations include aphthous stomatitis,<sup>42</sup> arthritis,<sup>43</sup> dental enamel defects,<sup>44</sup> and abnormal concentrations of liver transaminases.<sup>45</sup>

Coeliac disease might be diagnosed through screening of groups who have an increased risk of developing the disease, such as first-degree relatives of patients with coeliac disease and people with type 1 diabetes,<sup>46</sup> Down's syndrome,<sup>47</sup> or chronic liver disease, especially primary biliary cirrhosis.<sup>48</sup>

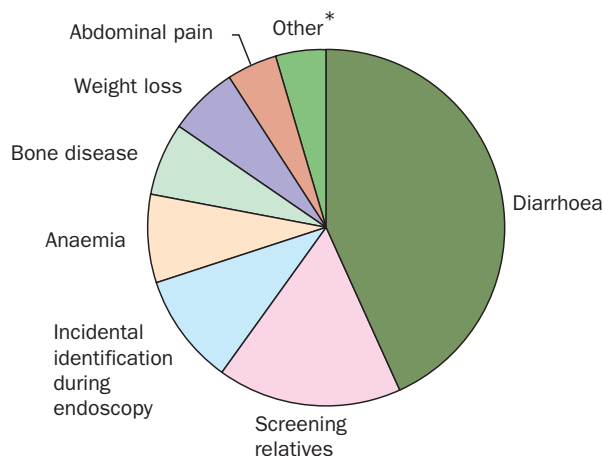


Figure 1: **Mode of presentation in 170 patients with coeliac disease**

\*Included dermatitis herpetiformis, peripheral neuropathy and constipation.

### Panel 1: Manifestations of non-gastrointestinal predominant (silent) coeliac disease

#### Children

Short stature  
Anaemia  
Neurological symptoms

#### Adults

Dermatitis herpetiformis  
Anaemia  
Reduced bone density  
Infertility  
Irritable bowel syndrome  
Dyspepsia  
Oesophageal reflux  
Neurological symptoms  
Autoimmune diseases

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## Diagnosis

Coeliac disease can be diagnosed in the presence of characteristic changes in a small intestinal biopsy sample and improvements in clinical symptoms or histological tests on a gluten-free diet.<sup>49</sup> Positive serological tests lend support to a diagnosis of coeliac disease, but they are not essential.

An intestinal biopsy might be done in several circumstances: if results of serological analysis are suggestive of coeliac disease; or if serological tests are negative, but clinical suspicion is high. Biopsies might also be done during endoscopy for any reason if signs of villous atrophy are evident, or if there is another indication of the disease, such as in the presence of iron deficiency anaemia, despite a normal endoscopic appearance (figure 2).

The major histological feature suggestive of coeliac disease is villous atrophy with crypt hyperplasia and intraepithelial lymphocytosis. However, there is a range of histological changes from normal villous architecture with an epithelial lymphocytosis, through partial villous atrophy to total villous atrophy.<sup>2</sup> In adults, although histological abnormalities can improve with a gluten-free diet, they often do not return to normal.<sup>50</sup>

## Serological testing

Serological tests have an important role in the management of patients with coeliac disease (panels 2 and 3) and provide the greatest chance of establishing the diagnosis of coeliac disease.

The presence of antibodies against endomysium is almost 100% specific in the diagnosis of coeliac disease.<sup>51</sup> The detection of such antibodies needs indirect immunofluorescence with either monkey oesophagus or human umbilical cord as the substrate. This fact, combined with the discovery that the autoantigen for the endomysial antibodies is the enzyme tissue transglutaminase<sup>52</sup> has led to the development of enzyme-linked immunoabsorbent assays that use either guineapig-tTG<sup>53</sup> or human-tTG.<sup>54</sup> As a result, in the USA when a test for endomysial antibodies is requested from commercial laboratories, one for anti-tissue transglutaminase antibodies (anti-tTG) is often done.

## HLA typing

Because more than 98% of people with coeliac disease share the major histocompatibility complex II class HLA-DQ2 or HLA-DQ8 haplotype, the inclusion of HLA typing for these haplotypes is useful, especially in patients with equivocal biopsy results or negative serological tests, or for patients already on a gluten-free diet. People who do not have HLA-DQ2 or HLA-DQ8 haplotypes are unlikely to have coeliac disease.<sup>55</sup>

## Pitfalls in the diagnosis of coeliac disease (panel 4)

### Serological tests

Selective IgA deficiency occurs in 1.7%–2.6% of patients with coeliac disease, a rate ten to 16-fold higher than that in the general population.<sup>56</sup> Individuals with selective IgA deficiency and coeliac disease will not have IgA antibodies (endomysial antibodies, anti-tTG, and AGA [antigliadin antibodies] IgA), but usually have a raised concentration of

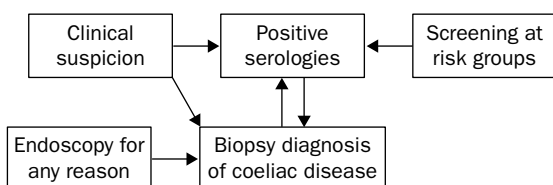


Figure 2: **Diagnosis of coeliac disease**

## Panel 2: Role of serological testing in patients with coeliac disease

Identification of patients for whom biopsy might be warranted  
Monitoring of adherence to the diet  
Screening of groups who are at risk of the disease

IgG antibodies. To detect coeliac disease in selective IgA deficient individuals, total serum IgA needs to be included in the panel of tests. Furthermore, a test for IgG antibodies (either IgG endomysial antibodies, IgG anti-tTG, or AGA IgG)<sup>57</sup> should be done. The combination of a positive IgG antibody test and IgA deficiency should prompt a biopsy.

The amount of gluten consumed by patients will alter the results of serological analysis, as will the use of immunosuppressants. Another pitfall of serological testing is that not all cases of coeliac disease are detectable by this method. Titres of endomysial antibodies correlate with the degree of villous atrophy<sup>58</sup> and presentation with symptoms.<sup>59</sup> As a result, patients with partial villous atrophy might not have antibodies against endomysium or tTG but will usually have antibodies against gliadin. Initial studies that showed high sensitivity of the test for endomysial antibodies<sup>60,61</sup> did not include patients with partial villous atrophy or were affected by preselection bias. Furthermore, the studies showing high sensitivity of the test for IgA antibodies against tTG did not include many patients who were negative for endomysial antibodies.<sup>53,62</sup> Therefore, reliance on antibodies against endomysium<sup>63,64</sup> or tTG<sup>65</sup> as a single test will underestimate the prevalence of coeliac disease by at least 20%. Furthermore, despite the fact that tTG is the autoantigen for endomysial antibodies, there is not always concordance between antibodies against endomysium and tTG,<sup>65-67</sup> which indicates the usefulness of combining antibodies against both endomysium and tTG for up to a third of patients will have only one of these antibodies.<sup>66</sup>

A negative anti-tTG might be a false negative because of a lack of sensitivity of the test. Most studies have been done with guineapig-tTG. An ELISA with human-tTG, either recombinant human-tTG<sup>64</sup> or human-tTG isolated from red blood cells<sup>68</sup> is now available and has greater sensitivity than the guineapig-tTG assay.<sup>67</sup>

Another difficulty is the low specificity of a test for anti-tTG in patients with chronic liver disease and in people with diabetes.<sup>69,70</sup> Additionally, a within laboratory comparison study, done with commercial laboratories in the USA, revealed a surprisingly low sensitivity for both AGA and endomysial antibodies,<sup>71</sup> indicating technical problems with these assays, a lack of standardisation, and differences in results between research and commercial laboratories.

Titres of IgA AGA and endomysial antibodies fall while on a gluten-free diet. Titres of endomysial antibodies are usually undetectable after 6–12 months, but can take up to 31 months if the initial titres are high.<sup>58</sup> Seroconversion precedes improvement in intestinal morphology.<sup>58</sup>

## Histological analysis

A major pitfall in the diagnosis of coeliac disease is in pathological interpretation of intestinal biopsies. An adequate number of biopsies needs to be taken because the disease is patchy and not all biopsy pieces will be adequately

## Panel 3: Serological tests used to diagnose coeliac disease

Antibodies against gliadin (IgA-AGA, IgG-AGA)  
Endomysial antibody (IgA-EMA)  
Tissue transglutaminase antibody (IgA-tTG)  
Total IgA

## Panel 4: Pitfalls in the diagnosis of coeliac disease

Problem	Effect	Solution
Selective IgA deficiency	Negative IgA-AGA, EMA & tTG	Include IgG antibody (AGA, tTG, EMA) and total IgA level. Biopsy if positive IgG antibody and IgA deficient
Partial villous atrophy	Negative EMA, tTG, or both	Include IgA-AGA in panel and biopsy if positive or if clinical suspicion is high, review biopsy. Add HLA-DQ2 and HLA-DQ8 testing
Lack of concordance between EMA and tTG	Negative EMA or tTG	Include both EMA and tTG in panel of tests
Poor specificity of GP-tTG in liver disease and diabetes	False positive tTG	Use assays with H-tTG as the antigen
Low gluten intake	Negative serologies	Gluten challenge before biopsy
Use of immunosuppressants	Negative serologies	Biopsy if clinical impression is high
Inter-laboratory variability in test results	False-negative results	Standardisation of tests
Patchiness of villous atrophy	Normal biopsy report	An adequate number of biopsies should be taken
Poor orientation of biopsy pieces	False interpretation of villous atrophy	Attempt orientation of biopsies. Order deeper sections of biopsy if clinical suspicion is high
Questionable diagnosis of coeliac disease	Unclear how to advise patient	Review biopsy, add HLA-DQ2/HLA-DQ8 testing, consider gluten challenge

GP-tTG=Guineapig tTG. H-tTG=human tTG.

oriented for interpretation of the crypt to villous ratio. A poorly oriented biopsy, as shown by crypts cut tangentially, will falsely reduce the crypt to villous ratio.<sup>72</sup> As a rule, at least three well oriented crypt to villous units need to be identified to interpret villous atrophy. Negative serological tests or poor response to the diet should prompt review of the biopsy to ensure that the original interpretation was correct.

### Pathogenesis and implications for innovative treatment

Coeliac disease is a T-cell mediated chronic inflammatory bowel disorder with an autoimmune component.<sup>3</sup> Loss of tolerance to gluten is a possible cause. Although such loss of tolerance has been identified in people without the disease, as manifested by the presence of peripheral blood T cells that respond to gluten,<sup>73</sup> the development of an anti-gluten T-cell response in the intestine is specific to people with coeliac disease.<sup>3</sup> The reason for this occurrence is obscure; however, changes in intestinal permeability secondary to alterations in intercellular tight junctions or in the processing of gluten are potential mechanisms.<sup>3,74</sup>

The immune response to gliadin takes place in two compartments, the lamina propria and the epithelium. While the part played by lamina propria CD4 T cells in pathogenesis is recognised, the role of CD8 T cells in the intestinal epithelium (intraepithelial lymphocytes) is controversial.<sup>3,75</sup>

#### Lamina propria

The role of lamina propria CD4 T cells is lent support by the strong genetic linkage to HLA-DQ2 and HLA-DQ8 and the identification of DQ2 and DQ8 anti-gluten restricted T cells that secrete interferon  $\gamma$ .<sup>3</sup> The discovery that gliadin is an excellent substrate for tTG,<sup>52</sup> a calcium dependent enzyme that transforms positively charged glutamines to negatively charged glutamic acid residues by deamidation,<sup>52</sup> led to the recognition that anti-gluten CD4 T cells in adults were mainly directed against deamidated peptides.<sup>76,77</sup> Additionally, specific gliadin peptides were noted to be immunodominant in adults<sup>78,79</sup>—ie, they were recognised by most anti-gluten CD4 T cells. These immunodominant peptides are part of a 33mer gliadin peptide that is resistant to digestion by gastric and pancreatic enzymes.<sup>80</sup>

One model is that deamidation of gliadin will create negative charges that are critical for the initiation of the immune response by allowing binding of gliadin peptides to the DQ2 or DQ8 grooves that have positively charged binding pockets (figure 3). Intestinal CD4 T cells that

recognise deamidated peptides presented by DQ2 and DQ8 produce interferon  $\gamma$ , which in turn provokes inflammation and villous atrophy.<sup>3</sup> Panel 5 shows the implications of these findings for the treatment of coeliac disease.

However, several observations raise questions about these potential treatments. The diversity of the antigluten response in children<sup>81</sup> lends support to an argument against the use of wheat in which the immunodominant peptides have been deleted, because it suggests that the antigluten immune response can be directed against a large panel of gliadin peptides. Furthermore, whether, and how, tTG plays a part in the pathogenesis of coeliac disease remains to be tested. Studies based on the characterisation of anti-gluten T cells in humans do not address this question because most gluten peptides presented to T cells in the intestine are deamidated by tTG and therefore only T cells that recognise deamidated peptides will expand. There is, in fact, evidence that deamidation is not necessary to induce the antigluten response. Native gluten (non-deamidated) peptides are preferentially recognised by peripheral T cells in adults and, at least in children, native peptides can be preferentially recognised by CD4 T cells.<sup>77,81</sup> Therefore, how necessary the deamidation process is for the development of an anti-gluten response is unclear.

Finally, the efficiency of oral peptidases is questionable.<sup>80</sup> First, other epitopes than the epitopes present in the 33mer have been shown to be toxic.<sup>81</sup> Second, knowing that only 100 mg per day of gluten is sufficient to induce mucosal damage, the oral peptidases would need to digest 96% of the 3000–4000 mg of gluten that is present in one sandwich. Such a digestion rate is unlikely, considering that peptidases would need to diffuse quickly in a semi-solid food mixture.

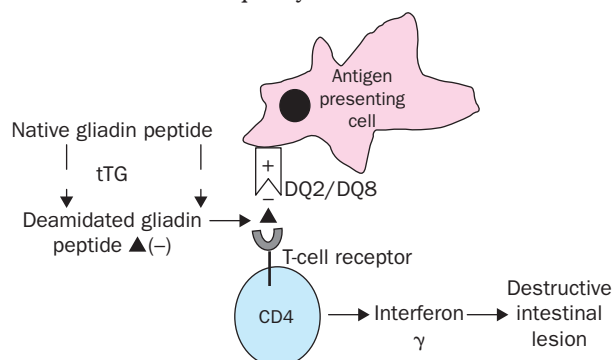


Figure 3: Model for anti-gliadin CD4 T-cell response in the lamina propria

tTG=tissue transglutaminase.

## Panel 5: Implication of research findings on treatment of coeliac disease

Research findings	Implications for treatment	Issues
Immunodominant peptides	Genetically modified wheat lacking these peptides. Oral peptidases	Presence of many epitopes, suggesting that modified gluten will remain toxic. Will enough epitopes be destroyed to prevent an antigluten response?
The CD4 T cell response is directed against peptides deamidated by tTG	Drugs blocking intestinal tTG	Is deamidation of gliadin essential for immunogenicity? What is the effect of blocking tTG in other tissues?
The CD4 T cell response is HLA-DQ2/HLA-DQ8 restricted	Peptides blocking the DQ MHC groove	Can these peptides inhibit a continuing pathogenic anti-gliadin response?
Interleukin 15 is upregulated, activates IEL, and favors development of lymphoma.	Use of anti-interleukin 15 antibodies in refractory sprue	Are anti-interleukin 15 antibodies sufficient to block epithelial lesions? Effect on viral infections?

## The epithelium

Intraepithelial lymphocytosis is a hallmark of coeliac disease and its importance is shown by the major complications of the disease—refractory sprue and enteropathy-associated T-cell lymphoma—which represent expansions of abnormal intraepithelial lymphocytes. The intraepithelial infiltration by CD8 T cells was thought to be secondary to the CD4 T-cell response in the lamina propria because no antigliadin-restricted intraepithelial lymphocytes could be identified. However, marked infiltrations by such lymphocytes do not exist in other intestinal disorders that are associated with an inflammation of the lamina propria, such as Crohn's disease and autoimmune enteropathy.<sup>75</sup> This finding indicates that the activation of lamina propria CD4 T cells cannot explain the expansion of intraepithelial lymphocytes in coeliac disease.

T-cell-mediated immune responses are not only directed against peptides, but can be aimed at recognising damaged cells that express molecules (MIC and HLA E) induced by stress and interferon gamma. Such alterations of the intestinal epithelium in response to gluten has been suggested in coeliac disease.<sup>82,83</sup> MIC and HLA E are recognised by the natural killer receptors NKG2D and CD94 present on intraepithelial lymphocytes that are upregulated by IL15.<sup>84–87</sup> Epithelial lesions could be accounted for by dysregulation of this system of stress and damage recognition in presence of high concentrations of interleukin 15, which arise in patients with coeliac disease. Upregulation of activating natural killer receptors by

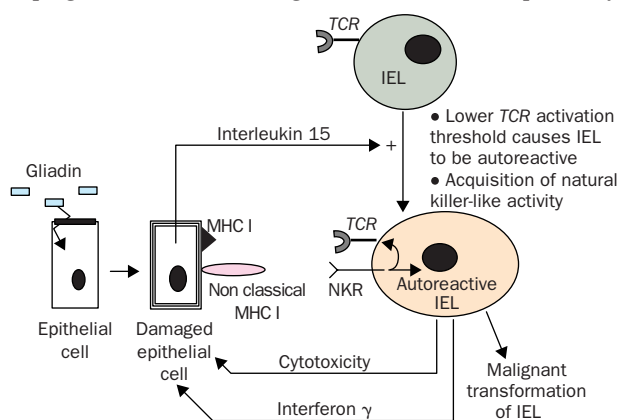


Figure 4: Role of intraepithelial lymphocytes in the pathogenesis of coeliac disease.

IEL=intraepithelial lymphocytes. MHC=major histocompatibility complex. NKR=natural killer receptor. In the presence of gluten, coeliac epithelial cells are altered, secrete interleukin 15, and express non-classical MHC class I molecules. In turn, interleukin 15 upregulates natural killer receptors in intraepithelial lymphocytes, which recognise non-classical MHC class I molecules. This leads to the activation of intraepithelial lymphocytes through two mechanisms: (1) the reduction of the activation threshold of intraepithelial lymphocytes and the recognition of low-affinity self-antigens; (2) the acquisition of natural killer-like activity. Activated intraepithelial lymphocytes destroy the epithelium by cytolysis and interferon  $\gamma$  secretion.

interleukin 15<sup>85</sup> could lead to uncontrolled activation of intraepithelial lymphocytes and villous atrophy (figure 4).

In this model, intraepithelial lymphocytes do not have to directly recognise gliadin peptides. However, a primary defect is implied in the epithelial cells of coeliac patients that would result in an abnormal sensitivity to gluten and the hypersecretion of interleukin 15—this is yet to be confirmed. An implication for treatment is the use of antibodies against interleukin 15 in refractory sprue, because interleukin 15 activates intraepithelial lymphocytes and promotes the development of lymphoma.<sup>85,88</sup>

## Treatment

The current treatment for coeliac disease is a strict gluten-free diet for life (panel 6), although there is the possibility of alternate treatments. The diagnosis should be definitive (ie, proven by biopsy) and a trial of gluten restriction, without a biopsy, should be avoided. In the gluten-free diet wheat, barley, and rye are avoided. Oats are not toxic to patients with coeliac disease or dermatitis herpetiformis.<sup>89,90</sup> However, there is reluctance in some countries to advise liberal use of oats because of the difficulty in guaranteeing that commercially available oats are free of contamination with other grains.

Most patients have a rapid clinical response to a gluten-free diet, although the rate of response does vary. Patients who are extremely ill might require admission, repletion of fluids and electrolytes, intravenous alimentation, and, occasionally, steroids. Patients should receive iron or folate supplementation if a deficiency in these minerals is documented.

Patients should also have a consultation with a dietitian who is knowledgeable about gluten-free diets.<sup>51</sup> However, not all dietitians are familiar with the intricacies of a gluten-free diet, and for this reason local or national support groups provide most of the required information.<sup>30</sup>

For adults, quality of life is improved on a gluten-free diet<sup>30</sup> even for those whose disease was detected by screening.<sup>91</sup> Children on a gluten-free diet reported a quality of life comparable to that of a reference population.<sup>92</sup> However, some investigators<sup>93</sup> have reported that women with coeliac disease failed to attain the same degree of subjective health as the general population. Furthermore, adolescents have difficulty with dietary compliance.

## Poorly or non-responsive coeliac disease

Some patients have a poor clinical or histological response to a gluten-free diet. An important first step in the assessment of these patients is to confirm the diagnosis of coeliac disease by review of the original biopsy, preferably by an expert in gastrointestinal pathology<sup>72</sup> (panel 7). However, the most common cause of a poor response is continued gluten ingestion, which might be intentional or unintentional. Therefore a consultation with an expert dietician is essential. Another reason for a poor clinical

### Panel 6: Initial management of patients with coeliac disease

Referral to dietician and support group  
 Screen for iron and folate deficiency  
 Ensure all regular medications are free of gluten  
 Advise bone density tests  
 Assess vitamin D and parathyroid hormone concentrations if osteoporotic  
 Advise serological screening of first-degree relatives

response to the diet might be lactose or fructose intolerance: intolerance to other foods is rare.

Treatments that might be of benefit include pancreatic supplements because of a reversible pancreatic insufficiency,<sup>94</sup> and a course of oral antibiotics in the case of coexisting bacterial overgrowth. Colonoscopy should be done, for biopsy, to exclude microscopic colitis,<sup>95</sup> collagenous colitis, or inflammatory bowel disease. Other causes of failure to respond to a gluten-free diet include autoimmune enteropathy characterised by positive antibodies against enterocytes,<sup>96</sup> lymphoma, ulcerative jejunitis, collagenous sprue, or refractory sprue.

The diagnosis of refractory sprue is considered in patients with coeliac disease who have intractable diarrhoea, villous atrophy, and failure to respond to a gluten-free diet. This might occur at presentation or after an initial response to a gluten-free diet.<sup>97</sup> Cellier and colleagues<sup>98</sup> have characterised these patients by analysing the phenotype of intraepithelial lymphocytes. The intraepithelial lymphocytes, although cytologically normal, expressed intracytoplasmic CD3 but did not have surface CD8, CD3, and TCR $\alpha\beta\gamma\delta$  expression. Because immunohistochemical studies do not distinguish intracellular and surface CD3 expression, the immunohistological phenotype of these intraepithelial lymphocytes is CD3+, CD8-,<sup>99</sup> whereas normal intraepithelial lymphocytes are CD3+, CD8+. Furthermore, these intraepithelial lymphocytes were noted to have an oligoclonal TCR $\gamma$  gene rearrangement.<sup>98</sup> The diagnosis of refractory sprue related to coeliac disease can, therefore, be established by immunohistochemical studies and demonstration of oligoclonal TCR gene rearrangement. However, even within patients with coeliac disease related refractory sprue other phenotypes are found, indicating this is a heterogeneous entity. For example, we have identified a patient with refractory sprue in whom oligoclonal intraepithelial lymphocytes were CD4+, CD56+.

There are reports of patients responding to steroids, azathioprine or ciclosporin,<sup>100,101</sup> and one to infliximab.<sup>102</sup>

### Panel 7: Causes of poorly responsive coeliac disease

Wrong diagnosis  
 Gluten ingestion\*  
 Lactose intolerance\*  
 Pancreatic insufficiency\*  
 Microscopic colitis\*  
 Bacterial overgrowth  
 Other food intolerances (fructose, milk, soy)  
 Collagenous colitis  
 Inflammatory bowel disease  
 Collagenous sprue  
 Ulcerative jejunitis  
 Enteropathy-associated T-cell lymphoma  
 Autoimmune enteropathy  
 Refractory sprue (with or without clonal T-cell populations)

\*Quite common.

Most patients in these reports have not had immunohistological phenotyping nor studies for TCR gene rearrangement. Therefore, whether these patients have refractory sprue associated with clonal TCR gene rearrangements, a group with a poor response to immunosuppressive therapy is unclear.<sup>103</sup>

The presence of phenotypically abnormal intraepithelial lymphocytes with clonal T-cell rearrangements has led several investigators to suggest that refractory sprue is a first step of malignant transformation leading to an enteropathy-associated T-cell lymphoma.<sup>98,104</sup> This finding is lent support by the progression of some patients with refractory sprue and ulcerative jejunitis to lymphoma.<sup>105</sup> Enteropathy-associated T-cell lymphoma is a non-homogeneous disease composed of at least two distinct forms. The main form, CD4-CD8-, with intracellular CD3+ is compatible with an origin from typical refractory sprue. However, the less common form of enteropathy-associated T-cell lymphoma characterised by CD3+CD8+CD56+ is not.<sup>106,107</sup>

Immunohistologic phenotyping and TCR gene rearrangement studies can be done with fixed tissue sections in non-specialised pathology laboratories. The use of these techniques in refractory patients and those with enteropathy-associated T-cell lymphoma will allow classification and gathering of data as to the frequency of these rare conditions and hopefully allow controlled studies of innovative treatments.

### Special considerations in coeliac disease

#### Malignant disease

Malignant diseases that are more frequent in patients with coeliac disease include small bowel adenocarcinoma, oesophageal and oropharyngeal squamous carcinoma, and non-Hodgkin lymphoma.<sup>108</sup> A gluten-free diet is thought to be protective against the development of malignant disease,<sup>109</sup> although this might not be the case for the development of non-Hodgkin lymphoma.<sup>110</sup>

Patients with coeliac disease have a risk of small bowel adenocarcinoma that is about 80-fold greater than that of the general population.<sup>30,111</sup> The predominant coeliac-associated lymphoma is the enteropathy-associated T-cell lymphoma, which does not respond well to chemotherapy and is rapidly fatal.<sup>112</sup> However, extra-intestinal lymphomas and B-cell lymphomas occur in patients with coeliac disease.<sup>113</sup> Results from a multicentre Italian study<sup>110</sup> suggest that coeliac disease is only a moderate risk factor for the development of non-Hodgkin lymphoma.

#### Osteoporosis

Measurement of bone mineral density is recommended when coeliac disease has been diagnosed because reduced bone density is common in both adults<sup>114</sup> and children with the disease.<sup>115</sup> The reduction in bone density is more severe in symptomatic coeliac disease than in the silent form<sup>116</sup> and is associated with an increased risk of fracture.<sup>117</sup> Bone mineral density improves after a gluten-free diet, but might not attain the normal range.<sup>118</sup>

The mechanism underlying osteoporosis is multifactorial, and might include calcium malabsorption, secondary hyperparathyroidism, vitamin D malabsorption, failure to obtain maximum bone density in childhood, magnesium deficiency, autoimmunity, the presence of circulating inflammatory cytokines, and reduced gonadal function in men.

Serologic screening of patients with idiopathic osteoporosis identifies patients with coeliac disease,<sup>119,120</sup> despite the absence of laboratory evidence of calcium malabsorption.<sup>120</sup>

## Fertility

Coeliac disease is associated with delayed menarche, premature menopause, amenorrhoea, recurrent abortions, and fewer children because of relative infertility before the diagnosis of coeliac disease<sup>121</sup> and reduced frequency of sexual intercourse.<sup>122</sup> Patients with coeliac disease are also reported to have babies with low birthweight,<sup>123</sup> increased perinatal mortality,<sup>124</sup> and a shorter duration of breast feeding.<sup>125</sup> Adherence to a gluten-free diet is associated with reduction in these poor outcomes. Coeliac disease might have clinical manifestations for the first time during pregnancy or puerperium.<sup>126</sup> Undiagnosed coeliac disease has been detected in infertile women who were screened for the disease,<sup>127,128</sup> but not in all studies.<sup>129</sup>

Infertility in men is also associated with coeliac disease.<sup>130</sup> Furthermore, men with the disease also tend to have children with a shorter gestation and lower birthweight than those without the disease.<sup>131</sup>

## Autoimmune disorders

Whether coeliac disease is an inflammatory disorder with secondary autoimmune reactions or whether it is a primary autoimmune disease induced by a known exogenous factor remains unclear. Autoimmune disorders arise ten times more often in patients with coeliac disease than they do in the general population. Such disorders include insulin dependent diabetes,<sup>46</sup> thyroid disease,<sup>132</sup> Sjögren's syndrome,<sup>133</sup> Addison's disease,<sup>134</sup> autoimmune liver disease,<sup>135</sup> cardiomyopathy,<sup>136</sup> and neurological disorders. When both autoimmune disease and coeliac disease occur in a patient, coeliac disease is frequently silent and as a result the autoimmune disorder is usually diagnosed first.

The association of autoimmune disorders and coeliac disease is thought to be related to a shared genetic tendency (HLA alleles) and a common immunological mechanism in addition to the presence of coeliac disease itself. There are several lines of evidence that lend support to the role of coeliac disease as a causative factor for autoimmune diseases. Some data suggest that the prevalence of autoimmune diseases was closely related to the duration of gluten exposure (ie, age at diagnosis of coeliac disease)<sup>137</sup> with children diagnosed before the age of 2 years having no increased frequency of autoimmune diseases. Additionally, patients with coeliac disease have a high rate of organ-specific autoantibodies at diagnosis compared with those on a gluten-free diet.<sup>138</sup> Furthermore, diabetes-related and thyroid-related serum antibodies can disappear when children with coeliac disease maintain a gluten-free diet.<sup>139</sup> Several autoimmune diseases might improve on a gluten-free diet, including neurological,<sup>140</sup> cardiac,<sup>141</sup> and renal diseases.<sup>142</sup> Results from these studies suggest that autoimmune diseases might be prevented by early diagnosis and treatment of coeliac disease and that in those with established autoimmune disorders, a gluten-free diet could offer a chance of improvement in symptoms.

## Conclusion

Coeliac disease is a very common disorder and most people with the disease have the silent form. Many of these patients are identified through screening of at-risk groups. The effect of the disease on the health of those with silent coeliac disease is unclear; however, it might account for several chronic health issues including osteoporosis, infertility, autoimmune diseases, and the worsening of other serious medical conditions. Identification of genetic and environmental factors and the understanding of the mechanisms that trigger the abnormal immune response to gluten will help us to understand the rationale for the wide clinical and histological spectrum, and in the long term to

facilitate the design of alternative treatments to the current strenuous gluten-free diet.

## Conflict of interest statement

None declared.

## Acknowledgements

We thank Donald Kasarda for helpful discussions and critical review of the manuscript. P H R Green and B Jabri contributed equally to this Seminar.

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