Recent advances in coeliac disease

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ABSTRACT – Coeliac disease, or gluten-sensitive enteropathy, affects 1 in 100–200 people in the UK. The condition, which is exacerbated by wheat, rye, barley and possibly oats, can be treated with a gluten-free diet in which these cereals are omitted. Serological screening, particularly of high-risk groups, with both IgA and IgG based systems can be used to identify cases. Diagnosis depends on the use of a small intestinal biopsy, which reveals the classical changes of loss of the normal villous architecture. Evidence suggests that gluten-sensitive T cells are involved in the pathogenesis of the disease. Use of *in vitro* systems has suggested an immuno-dominant epitope within wheat gliadin, which has been shown to exacerbate the condition *in vivo*. This information can be used to devise strategies to develop immuno-modulatory peptides and cereals with the baking and nutritional qualities of wheat, rye and barley, but which do not exacerbate the condition.

KEY WORDS: coeliac disease, gluten, immunodominant epitopes, pathogenesis, serological screening

Coeliac disease was known to the Romans, and the name itself derives from a description by Aretaeus the Capadocian, in the second century AD. He noted that the condition was associated with a swollen abdomen, hence the name coeliac disease, derived from the word *coelom* meaning the body cavity. The condition was first formally described by Samuel Gee in 1888 in his manuscript in the St Bartholomew's Hospital reports. He described it as a wasting condition, predominately affecting children, with diarrhoea resulting in weight loss, immaciation and ultimately death.[1] The disorder at that time was noted to have a 20% mortality rate. Samuel Gee reported that the condition must be cured by means of the diet and, indeed, had noted that a child thrived wonderfully on a diet of nothing but mussels. However, the child reported that he would rather die than continue to eat mussels for a second season.

Coeliac disease remains under-diagnosed. In recent years, its prevalence in Europe has increased over the past 20 years from 1 in 1800 to 1 in 300,[2–4] and it may affect 1 in 100–200 of the UK population.[5] This high prevalence is important as, without screening the whole population, it is essential to make efficient use of available resources by screening high-risk groups. For example, in patients with type I diabetes mellitus, the prevalence of coeliac disease is 5.7%.[6] Undiagnosed coeliac disease probably affects between 15 and 27% of patients with lymphocytic colitis.[7,8] A further 2% of patients with chronic fatigue syndrome and 3% of primary care patients with non-specific symptoms such as fatigue, irritable bowel syndrome, diarrhoea or unexplained anaemia are affected by the condition.[9]

The importance of early diagnosis and the introduction of a gluten-free diet has been underlined by a prospective study in which Corrao *et al*[10] studied 1,072 consecutive coeliac patients with a mean follow-up time of six years. The standardised mortality ratio (SMR) was 2.0 (95% CI 1.5–2.7) amongst patients with coeliac disease. Those who complied with a strict gluten-free diet had a good SMR of 0.5 (95% CI 0.2–1.1), whereas those whose adherence to a gluten-free diet was classified as poor had a considerably worse prognosis with an SMR of 6.0 (95% CI 4.0–8.9). Diagnostic delay was measured from the onset of symptoms to diagnosis by intestinal biopsy. An early diagnosis (diagnostic delay of <12 months) carried a much better prognosis (SMR = 1.5, 95% CI 0.9–2.3) than a medium delay (SMR 2.6, 95% CI 1.6–4.1) or a long delay (SMR 3.8, 95% CI 2.2–6.4). Most of the increased mortality among the coeliac patients is due to non-Hodgkin's lymphoma.

Key Points

The prevalence of coeliac disease in the UK is estimated to lie between 1 in 100 and 1 in 200 and the condition remains under-diagnosed

Serological screening with anti-tissue transglutaminase and anti-endomysial antibodies is both highly sensitive and specific for coeliac disease

HLA-DQ2 and DQ8 confer susceptibility together with as yet unidentified oligogenic loci

Gluten protein contains immunodominant epitopes that stimulate T cell responses in coeliac disease

Improved understanding of gluten epitopes opens up the possibility of developing novel coeliac non-toxic cereals and therapeutic altered peptide ligands

Screening tests

Several serological screening tests are available. They include anti-gliadin antibodies (both IgG and IgA class), and the anti-endomysial (EMA) IgA test. The latter has excellent specificity, approaching 100%,[11,12] although other groups have reported a lower sensitivity, if the test is used alone (74–93%).[12–14] Sensitivity partly reflects the problem of selective IgA deficiency among patients with coeliac disease, which affects 2–3% of sufferers, as this can cause false negative EMA IgA testing. Untreated coeliac disease patients with selective IgA deficiency commonly have positive IgG1 EMA and IgG tissue transglutaminase (tTG) antibodies (tissue transglutaminase being the antigen for endomysial antibodies). Therefore, if patients with selective IgA deficiency are not to be missed, an IgG-based test for coeliac disease needs to be included in screening programmes.[15]

Rapid bedside screening tests for coeliac disease, using a drop of blood from a finger prick and a 20-minute dot blot assay, can detect both IgA and IgG subtype tTG antibodies.[16] The sensitivity of the test is higher than IgA EMA testing alone: 70 out of 70 (100%) of the patients with untreated coeliac disease had positive results with the dot blot assay, and only 65 of 70 (92.8%) had a positive result for IgA EMA by standard indirect immunofluorescence.

Permeability tests

Intestinal permeability testing demonstrates impaired function of the small bowel mucosa, which can indicate untreated coeliac disease or concomitant disaccharidase deficiency. They are sensitive tests but are not specific to coeliac disease. As complementary tests in known coeliac patients, they can relate the presence of abnormal absorption and hence untreated disease. Additionally, permeability testing could potentially enable longitudinal study of individuals with latent coeliac disease – that is positive coeliac serology but normal duodenal histology – to determine whether they may develop coeliac disease.

Genetics

The majority of patients with coeliac disease carry either HLA-DQ2 or DQ8. The HLA-linked genes contribute only up to 40% of the genetic load.[17,18] The strong HLA association of coeliac disease with class II types DQ2 and DQ8 is now well established.

The only non-HLA locus to show reproducible evidence for association with coeliac disease between populations is the cytotoxic lymphocyte-associated (CTLA-4/CD28) gene region on 2q33. Association of markers within this region and coeliac disease has been separately demonstrated in French,[19] Swedish,[20] and Finnish[21] populations. A recent UK study demonstrated association of this region with susceptibility to coeliac disease.[22] CD28 and CTLA-4 molecules are expressed on lymphocytes and have a regulatory role on T cell function. CTLA-4 has an important role in maintaining tolerance to self-antigens. Indeed, severe autoimmune disease develops in CTLA-4-deficient mice.[23]

Genetic studies have also demonstrated an association of markers within the CTLA-4/CD28 region with other autoimmune diseases including Graves disease and type I diabetes mellitus.[24,25] It would therefore be tempting to postulate that a single mutation in the CTLA-4 gene or gene regulatory regions leads to an abnormality in CTLA-4 function or expression, and that this in turn leads to a general predisposition to autoimmune disease. However, this model does not fit well with the available evidence at the present time.

In summary, very exciting evidence of a susceptibility locus for coeliac disease on chromosome 2q33 has been presented. However, whether this locus is within the CTLA-4 gene or represents a separate nearby gene, and whether there is a single common susceptibility locus for several autoimmune diseases, remain to be determined.

Cereal chemistry

Coeliac disease is exacerbated by wheat, rye, barley and possibly oats. The active fraction in wheat is gliadin or the ethanol-soluble prolamin fraction. Several groups have studied gliadin to characterise the epitopes that exacerbate coeliac disease. *In vitro* studies have shown that several different gluten peptides are involved in the disease.[26–32] Most recently, two groups have presented evidence that the immunodominant gliadin epitope may lie within the region of amino acids 57–75 of alpha-gliadins.[33,34] These studies utilised peripheral blood T cells or isolated small intestinal T cell clones respectively.

HLA-DQ2 and HLA DQ8 molecules play a key role in the pathogenesis of the disease, by presenting peptides to antigen-specific T cells which promulgate the observed inflammatory response. Antigen-sensitive intestinal T cell clones to date have been DQ restricted.[35]

The only true test of toxicity is to confirm that these *in vitro* changes represent *in vivo* reactions in patients. We therefore investigated the effect of a peptide corresponding to amino acid residues 57–75 of alpha gliadin on the small intestinal mucosa of patients with coeliac disease who were receiving a gluten-free diet.[36] We compared the reaction produced to a positive control, a peptic-tryptic digest of gliadin (PTG) and a negative control peptide. We studied four adult patients with coeliac disease, all on a gluten-free diet. They all underwent three separate challenges. The peptides were instilled into the duodenum, and biopsies taken before the infusion and 2, 4 and 6 hours after commencing infusions. The results reveal that the negative control peptide caused no significant changes to the morphology or cellular infiltration of the small intestinal biopsies in any of the patients. However, 4–6 hours after commencing infusions, both PTG and the test peptide produced significant deterioration in the morphology of the biopsies, with lymphocytic infiltration, in all four subjects. Thus we concluded that the test peptide exacerbates coeliac disease *in vivo*.

This finding is important for a number of reasons. Firstly, we have demonstrated that the *in vitro* responses to the putative immunodominant epitope in alpha gliadin observed by other groups can be translated into *in vivo* toxicity. Secondly, based on

these studies, options are emerging for developing new therapies for the treatment of coeliac disease. Coeliac disease appears to be a Th1/ThO T cell mediated disease with antigenic stimulation producing secretion of the cytokine IFN-gamma. The immuno-dominant peptide could be produced with single amino acid residue substitutes, and these products could be tested in the future to investigate whether they could be used as altered ligands (APL) to induce T cell suppression by up-regulation of anti-inflammatory cytokines. Such peptides could be given as an oral preparation.

The finding also raises the possibility of producing non-toxic cereals by incorporating wheat DNA which not only lacks the toxic fragment of alpha gliadin, but could also incorporate blocking peptides, into a non-coeliac activating cereal such as maize or sorghum. This would allow the production of a new plant with the baking and nutritional of qualities of wheat, but which does not exacerbate coeliac disease.

Conclusion

Coeliac disease is a disorder which affects 0.5% of the population of the UK. It causes considerable morbidity, often presenting with diarrhoea, malnutrition and failure to thrive in infants, or diarrhoea and osteoporosis in later life. An increased use of serological screening tests, including IgA anti-endomysial and anti-tissue transglutaminase tests, have been used to show the higher true prevalence of the condition. The disease is thought to be mediated by gluten-sensitive T cells in the small intestinal mucosa. It has been proposed that peptides are presented by HLA DQ2 or DQ8 molecules to antigen-sensitive T cells which induce a Th1 inflammatory type response. Recent studies have permitted identification of immunodominant epitopes in this condition. This is important as this creates the possibility of therapeutic intervention.

Acknowledgements

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