ARCHIVES OF DISEASE IN CHILDHOOD

The Journal of the British Paediatric Association

Annotations

Molecular biology of coeliac disease

The 'toxic' antigen

Coeliac disease was first described by Samuel Gee in 1888.¹ He observed the remission of one individual on an exclusive diet of mussels, but it was not until 1952 that the link with dietary ingestion of certain cereals was made. Dicke observed the effect of bread rationing in Holland during the second world war, noting an improvement among children with coeliac disease who subsequently relapsed when fresh supplies were air dropped.² Since this time, studies have been undertaken to identify the component of wheat cereals that induces the coeliac lesion and to ascertain the nature of this susceptibility to dietary antigens.

Dicke extended his original observations through the purification of wheat flour and *in vivo* testing of subfractions. He concluded that the toxic component resided within the alcohol soluble wheat gliadin storage proteins, rather than the water soluble wheat starch, or the insoluble wheat glutenin storage proteins, and that this toxicity was lost by deamidation of the glutamine residues. Electrophoresis of gliadin has identified several classes of gliadin proteins, termed α , β , γ , and ω , although each subgroup is itself comprised of a variety of proteins. Toxicity has been shown to reside with all four classes of gliadins, although α -gliadins have the greatest *in vivo* effects.

To define further the toxic gliadin component, a single A-gliadin protein from the α -gliadin subclass has been purified and sequenced. This protein is 266 amino acids in length and can be subdivided into five domains, with domains II and IV being composed of polyglutamine sequences. A variety of *in vitro* tests, including jejunal organ culture from untreated coeliac patients and lymphocyte migration studies, have been used in an attempt to isolate a toxic sequence motif from A-gliadin. The majority of these studies have indicated that coeliac disease patients may be sensitised to a sequence within domain I between residues 3–56. Several reports have also suggested that an additional antigen motif may reside within domain V, between residues 206–217, by virtue of a shared sequence with residues 384–395 of the E1b protein of adenovirus 12. A hypothesis was proposed that prior exposure to adenovirus 12 may lead to sensitisation to this shared sequence in domain V. However, although an initial *in vitro* study suggested toxicity, a subsequent *in vivo* study of this sequence was inconclusive.^{3–5} Attempts by other investigators to demonstrate prior infection in newly diagnosed coeliac patients have been unsuccessful. No

humoral immunity is demonstrable against the E1b Ad12 sequence from the sera of coeliac patients. Finally, monoclonal antibodies raised against the 206–217 sequence do not recognise all toxic cereals, but will cross react with non-toxic cereals, implying that this sequence is not toxic to coeliac patients.⁶

Gliadin specific T cell clones have now been isolated from coeliac disease patients.⁷ The characterisation of one of these clones has identified a sequence from A-gliadin domain I, residues 31–49, as the T cell epitope.⁸ The sequence is unique to 'toxic' cereals, in that a monoclonal antibody raised against this sequence reacts specifically with α , β , γ , and ω gliadins as well as wheat, barley, rye and oats, but not with non-toxic cereals such as rice and maize.⁹ *In vitro* jejunal organ cultures from coeliac disease patients have confirmed toxicity.¹⁰ *In vivo* testing of this peptide sequence has been performed.¹¹ Four coeliac disease patients were subjected to gliadin challenge and assessed for enteropathic changes. Challenges were subsequently performed with A-gliadin peptide residue 31–49, as well as two alternative A-gliadin peptides – residue 202–220 from domain V and residue 3–21 from domain I. Jejunal biopsy specimens were assessed for intraepithelial lymphocyte count, enterocyte cell height, and villous height to crypt depth ratio from time 0 to 6 hours. The results demonstrated significant changes in all three parameters after challenges with both unfractionated gliadin and the peptide sequence 31–49, but no significant changes with the other two peptides. This *in vivo* study therefore confirms the results of *in vitro* experiments, demonstrating that patients with coeliac disease are sensitised to an antigen peptide from A-gliadin derived from the amino acid sequence residues 31–49.

Is this peptide sequence the only antigenic epitope among the gliadin proteins which will induce a small bowel inflammatory response in susceptible individuals? The *in vitro* studies performed up until now have concentrated on the characterised A-gliadin polypeptide. The sequences of other gliadin polypeptides remain undetermined. Several further gliadin specific T cell clones have now been isolated, although they have yet to be fully characterised.⁷ They do not appear to share a common T cell receptor motif. All are reactive to digested gliadin, but do not appear sensitised to the peptide sequence residues 31–49. While the identity of the peptide epitopes of these T cell clones is not yet established, it appears likely that multiple antigenic epitopes will exist within gliadin.

The isolation of gliadin specific T lymphocytes confirms the long suspected role of the cellular immune system in the initiation of the coeliac gliadin response. All T cell clones isolated are CD4 positive, and bear an α/β T cell receptor. Immunohistochemistry has previously indicated that the CD4 positive α/β T cells within the small bowel lamina propria display activation markers after dietary gliadin challenge. In vitro stimulation of these α/β T cells using superantigens has demonstrated their ability to mediate the small bowel lesion found in untreated coeliac disease.¹²

The role of a different subset of T lymphocytes bearing a γ/δ T cell receptor remains less well understood. The γ/δ T cells are located within the small bowel epithelium. Their numbers are increased in the untreated lesion, and remain raised above normal after resolution of the mucosal damage. Although previous hypotheses have suggested that these γ/δ T cells may have a primary role in the disease pathogenesis, gliadin specific γ/δ T cell lines have not been found.¹³ Studies of other T cell mediated small bowel inflammatory conditions, such as tropical sprue, protozoal and viral infections, have also demonstrated increases in intraepithelial γ/δ T cell numbers.¹⁴ It has been suggested that these γ/δ T cells may play a part in immune surveillance, through the recognition of heat shock proteins expressed on the enterocyte cell surface. An alternative hypothesis suggests that these γ/δ T cells are involved in the process of immunological tolerance seen in the gastrointestinal tract on exposure to luminal antigens. A study of γ/δ T cells in a mouse model implies that these cells may have the ability to abrogate the local development of oral tolerance, enabling the T cells located within the lamina propria to recognise antigen, and so regulate the plasma cell production of secretory IgA.¹⁵

Genetic susceptibility

Susceptibility to gluten sensitivity appears from family studies to be, at least in part, genetically determined. The incidence of coeliac disease in the first degree relatives of an affected individual has been estimated at 10–15%, while monozygotic twin data indicate a 70–100% disease concordance. The influence of the HLA genes has been well characterised both in family and population studies. The predisposition to the disease is closely associated with the inheritance of two alleles at the HLA-DQ loci, that encode for the α and β chains of a specific HLA class II molecule.¹⁶ These alleles are termed DQA1*0501 and DQB1*0201 respectively and they encode for HLA-DQ2 molecule. These alleles are found in over 90% of coeliac patients from northern and southern Europe. These HLA-DQ alleles are located at loci within the major histocompatibility complex (MHC) on chromosome 6. They are usually inherited together with alleles occurring at neighbouring loci, on what is termed an extended haplotype, and this common inheritance of several alleles has created difficulty in the identification of the primary susceptibility alleles.

In the UK, the HLA-DQ susceptibility alleles occur on the same chromosome as the HLA class II allele HLA-DR3 and HLA class I allele HLA-B8. Although both of HLA-DR3 and -B8 have previously been proposed as the primary association, the HLA-DQ alleles are now recognised as the primary HLA susceptibility influence towards coeliac disease after the study of southern European populations.

In Sardinia, differing HLA haplotypes are found compared with the UK. In particular, the HLA-‘DQ2’ alleles and HLA-DR3 are inherited with HLA-B18 rather than -B8, although this does not affect the close DQ2 association

with coeliac disease (all coeliac patients possess the alleles DQ2 and DR3).¹⁷ This would suggest that the primary susceptibility influence does not reside with HLA-B8.

In southern European regions, such as central Italy, the frequency of all HLA DR3-DQ2 haplotypes is low compared both with the UK and Sardinia. Coeliac disease in these populations is found associated not only with the DR3-DQ2 haplotypes, but also the heterozygous combination of HLA-DR5/-DR7 (that is, a DR5 haplotype on one chromosome and a DR7 haplotype on the other). At the DQ loci, however, coeliac disease remains strongly associated with the DQA1*0501 DQB1*0201 allele combination, encoding for the DQ2 molecule.¹⁸ This DQ2 association is due to the presence of the DQA1*0501 allele on the DR5 haplotype, and the DQB1*0201 allele on the DR7 haplotype. As neither the DR5 haplotype nor the DR7 haplotype is associated with coeliac disease in the absence of the other, it can be concluded that the primary susceptibility alleles reside at the DQ loci.

Role of HLA-DQ2 towards coeliac disease susceptibility

T cells are unable to recognise antigen directly, unless it is processed and presented to the T cell receptor in a specific manner. The HLA molecules are the antigen presenting system for T cells, and in particular the HLA class II molecules are responsible for the presentation of exogenous antigen to CD4+ve T cells. Coeliac disease is a sensitivity to exogenous gliadin antigen, mediated through CD4+ve T cells, and it has been hypothesised that the HLA-DQ2 molecule is necessary for the correct presentation of the disease inducing antigen to these gliadin reactive T cells.

The isolation of gliadin specific T cells has enabled this hypothesis to be tested. The initial results confirmed that HLA-DQ2 restricted gliadin specific T cells are present in patients with coeliac disease. Subsequent studies have found that all gliadin specific T cells isolated from the small bowel only recognise gliadin antigen in the presence of HLA-DQ2 and not -DR and -DP class II molecules.⁷ In peripheral blood, additional gliadin reactive T cells have been described that are restricted also to -DR and -DP molecules – both in coeliac patients and ‘normal’ controls.¹⁹ The significance of these additional T cells is not understood at present, but may indicate that the disease-inducing gliadin/HLA-DQ2/CD4+ve T cell interaction occurs predominantly within the gastrointestinal immune systems.

Antigen presentation by HLA-DQ2

The HLA-DQ2 molecule appears fundamental for the recognition of gliadin by T cells in the small bowel of coeliac patients. As many other HLA-DQ variants seem unable to mediate this interaction, detailed analysis of the mechanism of antigen binding may lead to further insight into the disease pathogenesis and potentially open the way to genetic modification of the gliadin antigen so as to abrogate the T cell recognition.

The structure of the HLA class II heterodimer has now been elucidated by x ray crystallography.²⁰ The tertiary structure of this molecule forms a groove of 40 Å in length on its upper surface into which antigen peptide is bound for presentation to the T cell receptor. Within this groove, pockets are sited to accept the amino acid side chains of the antigen peptide to enable binding. Polymorphic allelic variants encode for amino acid substitutions within this binding groove, predominantly at the location of these pockets, and thus affect the binding affinity of a particular HLA molecule for specific antigen peptides. Speculation

would suggest that the configuration of this antigen binding groove on the HLA-DQ2 molecule is important for the presentation of gliadin antigen in the induction of coeliac disease.

A second combination of HLA class II alleles has been found to influence susceptibility towards coeliac disease in eastern Mediterranean populations. Although the HLA-DQ2 alleles remain the predominant susceptibility genes in these regions, up to 20% of coeliac disease patients possess genes encoding an alternative DQ molecule, termed DQ8. The genes encoding DQ8 (DQA1*0301 DQB1*0302) are found on DR4 haplotypes.²¹ Gliadin specific T cells have been isolated from these patients and confirmed as restricted to the HLA-DQ8 molecule.⁵ The identification of a second HLA susceptibility molecule allows comparison to be made between the molecular structures of susceptible and non-susceptible genes.

The susceptible HLA-DQ8 and the non-susceptible HLA-DQ7 molecules differ by only four amino acid substitutions. Two of these substitutions, at positions β 13 and 26, are located in a side chain binding pocket in the floor of the antigen binding groove. These two substitutions are common to both HLA-DQ8 and HLA-DQ2 molecules, although HLA-DQ2 also possesses a unique combination of hydrophobic residues at a neighbouring pocket (β 37, 38, 45, and 58). Furthermore the α -chains of both HLA-DQ2 and -DQ8 uniquely possess a polar residue (α 44) at the floor of a third deep pocket at the far left hand end of the groove, rather than a positively charged amino acid found on all other DQ α chains. These findings suggest that the genetic susceptibility towards coeliac disease, derived from inheriting certain HLA alleles, reflects structural differences in the key antigen binding sites on the HLA molecule.

The next question to be answered is how substitutions at these key antigen contact points affect antigen binding affinity, specifically with gliadin peptides. Work is currently under way to examine the effects of point mutations on the binding of the gliadin A peptide, residues 31–49, to HLA-DQ2 and will hopefully provide the answers.

RICHARD TIGHE
PAUL J CICLITIRA

Department of Gastroenterology,
Rayne Institute,
St Thomas's Hospital,
UMDS, London SE1 7EH

- 1 Gee S. On the coeliac affection. *St Bartholomew's Hospital Report* 1888; **24**: 17–20.
- 2 Dicke WK. Coeliakie. Utrecht: University of Utrecht, 1950. (MD thesis.)
- 3 Kagnoff MR, Raleigh KA, Hubert JJ, Bernadin JF, Kasarda DD. Possible role of a human adenovirus in the pathogenesis of coeliac disease. *J Exp Med* 1984; **160**: 1544–7.
- 4 Mantzaris GJ, Karagiannis JA, Priddle JD, Jewell DP. Cellular hypersensitivity to a synthetic dodecapeptide derived from human adenovirus 12 which resembles a sequence of A-gliadin in patients with coeliac disease. *Gut* 1990; **31**: 668–73.
- 5 Mantzaris G, Jewell DP. In vivo toxicity of a synthetic dodecapeptide from A gliadin in patients with coeliac disease. *Scand J Gastroenterol* 1991; **26**: 392–8.
- 6 Ellis H, Doyle A, Sturgess R, Ciclitira P. Coeliac disease: characterisation of monoclonal antibodies raised against a synthetic peptide corresponding to amino acid residues 206–217 of A-gliadin. *Gut* 1992; **33**: 1504–7.
- 7 Lundin KEA, Scott H, Hansen T, et al. Gliadin-specific, HLA-DQ(α 1*0501, β 1*0201) restricted T cells isolated from the small intestinal mucosa of coeliac disease patients. *J Exp Med* 1993; **178**: 187–96.
- 8 Gjertsen HA, Lundin KEA, Sollid LM, Erikson JA, Thorsby E. T cells recognise a peptide derived from α -gliadin presented by the coeliac disease-associated HLA-DQ (α 1*0501, β 1*0201) heterodimer. *Hum Immunol* 1994; **39**: 243–52.
- 9 Ellis HJ, Doyle AP, Wieser H, Sturgess RP, Ciclitira PJ. Specificities of monoclonal antibodies to domain I of α -gliadins. *Scand J Gastroenterol* 1993; **28**: 212–6.
- 10 Shidrawi R, Day P, Przemioslo R, Ellis H, Nelufer J, Ciclitira P. In vitro toxicity of gluten peptides in coeliac disease assessed by organ culture. *Scand J Gastroenterol* 1995 (in press).
- 11 Sturgess R, Day P, Ellis H, et al. Wheat challenge in coeliac disease. *Lancet* 1994; **343**: 758–61.
- 12 Lionetti P, Spencer J, Breese EJ, Murch SH, Taylor JW, MacDonald TT. Activation of mucosal V β 3+ T cells and tissue damage in human small intestine by the bacterial superantigen Staphylococcus aureus enterotoxin B. *Eur J Immunol* 1993; **23**: 664–8.
- 13 Rust C, Kooy Y, Pena S, Mearin ML, Kluijn P, Koning F. Phenotypic and functional characterization of small intestinal TCR gamma delta + T cells in coeliac disease. *Scand J Immunol* 1992; **35**: 459–68.
- 14 Spencer J, Isaacson PG, MacDonald TT, Thomas AJ, Walker-Smith JA. Gamma/delta T cells and the diagnosis of coeliac disease. *Clin Exp Immunol* 1991; **85**: 109–13.
- 15 Fujihashi K, Taguchi T, Aicher W, et al. Immunoregulatory functions for murine intraepithelial lymphocytes: γ/δ T cells receptor-positive (TCR+) T cells abrogate oral tolerance, while α/β T TCR+ T cells provide B cell help. *J Exp Med* 1992; **175**: 695–707.
- 16 Sollid L, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of coeliac disease to a particular HLA-DQ α/β heterodimer. *J Exp Med* 1989; **169**: 345–50.
- 17 Coniga M, Frau F, Lampis R, et al. A high frequency of the A30, B18, DR3, DRw52, DQw2 extended haplotype in Sardinian celiac patients: further evidence that disease susceptibility is conferred by DQA1*0501, B1*0201. *Tissue Antigens* 1992; **39**: 78–83.
- 18 Tighe MR, Hall MA, Barbado M, Card E, Welsh KI, Ciclitira PJ. HLA class II alleles associated with coeliac disease susceptibility in a southern European population. *Tissue Antigens* 1992; **40**: 90–7.
- 19 Gjertsen HA, Sollid LM, Ek J, Thorsby E, Lundin KE. T cells from the peripheral blood of coeliac disease patients recognize gluten antigens when presented by HLA-DR, -DQ, or -DP molecules. *Scand J Immunol* 1994; **39**: 567–74.
- 20 Brown JH, Jardensky TS, Gorga JC, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 1993; **364**: 33–9.
- 21 Tighe MR, Hall M, Ashkenazi A, Siegler E, Lanchbury J, Ciclitira PJ. Coeliac disease among Ashkenazi Jews from Israel: a study of the HLA class II alleles and their associations with disease susceptibility. *Hum Immunol* 1993; **38**: 270–6.