

REVIEW

Coeliac disease: a unique model for investigating broken tolerance in autoimmunity

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Coeliac disease, a prevalent immune-mediated enteropathy driven by dietary gluten, provides an exceptional human model to dissect the genetic, environmental and immunologic factors operating in autoimmunity. Despite the causative antigen being an exogenous food protein, coeliac disease has many features in common with autoimmune disease including a strong HLA class II association and the presence of pathogenic CD4⁺ T cells and autoantibodies. CD8⁺ intraepithelial lymphocytes specifically target and destroy intestinal epithelium in response to stress signals and not a specific antigen. A unique feature of coeliac disease is the ability to remove gluten to induce disease remission and reintroduce it to trigger a memory response. This provides an unparalleled opportunity to study disease-relevant CD4⁺ T cells that have been expanded *in vivo*. As a result, the causative peptides have been characterised at a level unprecedented for any autoimmune disease. Despite the complexity of the gluten proteome, resistance to gastrointestinal proteolysis and susceptibility to post-translational modification by transglutaminase help shape a restricted repertoire of immunogenic gluten peptides that have high affinity for disease-associated HLA. The critical steps in coeliac disease pathogenesis have been broadly elucidated and provide the basis for experimental therapies in pre-clinical or clinical development. However, little is known about how and why tolerance to gluten sometimes breaks or fails to develop. Understanding the interactions between genes, the environment, gluten immunity and the microbiome may provide novel approaches for the prevention and treatment of disease.

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INTRODUCTION

Coeliac disease (CD) is a chronic immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals.¹ The seminal work of Dutch paediatrician Willem Dicke in the 1940s established a component of wheat, subsequently shown to be gluten, was the environmental driver of CD, and that removal of wheat from the diet led to prompt clinical recovery. The dietary trigger and prominent clinical phenotype of malabsorption influenced the view that CD is primarily a gastrointestinal illness. However, advances in the understanding of its genetic and immunologic basis now firmly position CD as an immune illness with systemic manifestations and features more in common with autoimmune disease (AID), where a pathogenic adaptive immune response targets self antigens. In common with many AID, genetic and environmental factors are important in CD development, inheritance is polygenic, a strong association with specific histocompatibility leucocyte antigen (HLA) genes exists, and both pathogenic CD4⁺ T cells and autoantibodies are present.² Circulating autoantibodies directed against the endogenous enzyme tissue transglutaminase 2 (TG2) are a feature of active CD, and notably, their formation is dependent on and driven by the exogenous antigen gluten. Anti-TG2 antibodies can be detected in the intestine before overt tissue damage occurs, and have several

pathogenic effects. Furthermore, recent insights into a key effector role for CD8⁺ intraepithelial lymphocytes (IELs) in the targeted killing of intestinal enterocytes that express IL-15 and stress-induced molecules has prompted some experts to consider this cell auto-reactive.²

Despite many similarities with AID, CD is unique in that the driving antigen, gluten, is exogenous. Several other features set it apart from other more 'classical' AID, including the ability to easily access and sample the main target organ (intestine) by endoscopy, and that disease-specific CD4⁺ T cells can be readily isolated from the intestine and blood following gluten ingestion. Furthermore, the HLA association in CD, one of the strongest of all human HLA-linked diseases, shapes a restricted repertoire of immunogenic gluten peptides. These features mean that gluten has been better characterised than any other antigen implicated as causative in AID, and also make CD an ideal model to dissect the genetic and immune pathways potentially relevant in AID pathogenesis. Here, we review the genetic, environmental and immunologic factors that contribute to broken tolerance to gluten and why CD is of significance to the AID field.

A GLOBAL CLINICAL PROBLEM ON THE RISE

CD affects 1–2% of the Western population and, like many chronic inflammatory diseases and AID, is substantially increasing in

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Table 1 Gene candidates and loci associated with coeliac disease and their overlap with autoimmune diseases

Gene candidate(s) and functional roles	Locus	Autoimmune disease associations ¹⁴	Refs
<i>RSBN1</i> : Suggested role in transcriptional regulation in haploid germ cells.	1p13.2	AA, ATD, Cro, JIA, RA, SLE, T1D, VIT	13
<i>PTPN22</i> : Implicated in T cell receptor signalling.			
<i>PHTF1</i> : May play a role in transcription regulation.			
<i>AP4B1-AS1</i> : RNA gene affiliated with the non-coding RNA class. Unknown function.			
<i>DCLRE1B</i> : Blocks transcription, replication, and segregation of DNA.			
<i>MAGI3</i> : Scaffolding protein at cell-cell junctions.			
<i>AP4B1</i> : Targeting proteins from the trans-Golgi network to the endosomal-lysosomal system.			
<i>NFIA</i> : Neurological tissue development. Myeloid cell differentiation.	1p31.3	AS, Cro, PBC, PS, UC	101
<i>RUNX3</i> : Thymic CD8 ⁺ T cell differentiation	1p36.11	AS, PS	10
<i>TNFRSF14</i> : Inflammatory signalling.	1p36.32	ATD, IBD, MS, PSC, RA, UC	10
<i>MMEL1</i> : Expressed in testis and CNS, likely involved in neuropeptide degradation.			
<i>FAM213B</i> : Catalyses the reduction of prostaglandin.			
<i>TTC34</i> : Unknown function.			
<i>FASLG</i> : Apoptotic signalling, T cell induced cell death.	1q24.3	Cro, IBD	12
<i>TNFSF18</i> : T cell survival.			
<i>NCF2</i> : Oxidative stress.	1q25.3	SLE	102
<i>SMG7</i> : Nonsense-mediated mRNA decay.			
<i>RGS1</i> : Intestinal intra-epithelial lymphocyte expression. Regulates chemokine receptor signalling and B cell activation and proliferation.	1q31.2	MS, T1D	11
<i>C1orf106</i> : Unknown function.	1q32.1	AS, Cro, MS, SLE, T1D, UC	10
<i>DUSP10</i> : Negative regulator of MAP kinases involved in cellular proliferation and differentiation.	1q41		103
<i>PUS10</i> : Modulator of TRAIL-induced cell death.	2p15-16.1	AS, Cro IBD, MS, PS, RA, UC	10,12
<i>PLEK</i> : Major protein kinase C substrate of platelets.			
<i>FBXO48</i> : Unknown function.			
<i>IL18R1</i> and <i>IL18RAP</i> : Induction of IFN- γ by T cells.	2q12.1	Cro, IBD, PBC	11
<i>ITGA4</i> : Adhesion, migration, and activation of immune cells.	2q31.3	AS	104
<i>UBE2E3</i> : Encodes ubiquitin conjugating enzyme which may play a role in cell proliferation.			
<i>STAT4</i> : T cell differentiation.	2q32.2-32.3	Cro, IBD, JIA, MS, PBC, RA, SJO, SLE, SSC, T1D, UC	12
<i>CD28</i> , <i>CTLA4</i> , and <i>ICOS</i> : T and B cell inhibitory/activatory receptors.	2q33.2	AA, ATD, PSC, RA, T1D	10
<i>CCR4</i> : Cell recruitment.	3p22.3	MS	10
<i>GLB1</i> : Hydrolyses beta-galactose from glycoconjugates.			
<i>CCR1</i> , 2, 3: Cell recruitment to inflamed sites.	3p21.31	Cro, JIA, T1D, UC	11
<i>LTF</i> : Encodes lactotransferrin, an iron-binding glycoprotein with broad innate immune effects.			
<i>ARHFP31</i> : Protein trafficking and cell growth.	3q13.33	MS, PBC, VIT	10
<i>SCHIP1</i> : Suggested functional connection with actin cytoskeleton and tumour suppression.	3q25.33	MS, PBC, SJO, SLE, SSC	11
<i>IL12A</i> : T cell and NK cell stimulation.			
<i>LPP</i> : Possible role in cell adhesion and motility.	3q28	ATD	11
<i>IL2</i> : T-cell activation and proliferation.	4q27	AA, Cro, IBD, JIA, PSC, RA, T1D, UC	6
<i>IL21</i> : B, T, NK cell proliferation and cytokine production.			
<i>KIAA1109</i> : Thought to regulate epithelial growth and differentiation.			
<i>ADAD1</i> : Testis expression profile. Plays a role in spermatogenesis.			
<i>HLA-DQA1</i> and <i>HLA-DQB1</i> : Antigen presentation to CD4 ⁺ T cells.	6p21	RA, T1D, SLE, MS, UC, PSC, AS, ATD, PS, IBD, Cro, AA, VIT, JIA, PBC, SJO	6,13,16
<i>HLA-DPB1</i> : Antigen presentation to CD4 ⁺ T cells.			
<i>HLA-B</i> : Antigen presentation to CD8 ⁺ T cells.			
<i>HLA-F</i> : HLA-F expressed on activated lymphocytes. Role unclear.			
<i>B3GALT4</i> : Ganglio-series glycolipid biosynthesis.			
<i>IRF4</i> : Transcription factor with role in regulating Toll-like receptor signalling and interferon production.	6p25.3	PS, RA	12
<i>BACH2</i> : T cell regulation, B cell differentiation, macrophage-mediated innate immunity.	6q15	ATD, Cro, MS, RA, T1D	10
<i>PTPRK</i> : Maintenance of cell junctions and proliferation. CD4 ⁺ T cell development.	6q22.33	Cro, IBD, MS, T1D	10
<i>TNFAIP3</i> : NF- κ B signalling.	6q23.3	IBD, MS, PBC, PS, RA, SJO, SLE, T1D, UC	105
<i>OLIG3</i> : Neuronal development.			
<i>TAGAP</i> : T-cell activation.	6q25.3	Cro, MS, PS, RA, T1D	11
<i>ELMO1</i> : Phagocytosis and cell migration.	7p14.1	MS, PS, RA	12
<i>PVT1</i> : Candidate oncogene.	8q24.21	Cro, IBD, MS, RA	10
<i>PFKFB3</i> : Cell cycle progression and prevention of apoptosis.	10p15.1	AA, ATD, Cro, IBD, JIA, MS, PSC, RA, T1D, VIT	12
<i>PRKCQ</i> : Kinase important for T-cell activation.			

Table 1 (Continued)

Gene candidate(s) and functional roles	Locus	Autoimmune disease associations ¹⁴	Refs
<i>ZMIZ1</i> : Transcriptional coactivator.	10q22.3	AS, Cro, IBD, MS, PS	10
<i>POU2AF1</i> : B cell development and function.	11q23.1	PSC	12
<i>C11orf93</i> : Unknown function.			
<i>TREH</i> : Intestinal trehalase is probably involved in the hydrolysis of ingested trehalose.	11q23.3	Cro, MS, PBC, RA	12
<i>DDX6</i> : Translation suppression, mRNA degradation, and microRNA-induced gene silencing.			
<i>ETS1</i> : Thymic CD8 ⁺ lineage differentiation.	11q24.3	PS, RA, SLE	10
<i>SH2B3</i> : Regulates T cell signalling.	12q24.12-24.13	AA, AS, JIA, PBC, PSC, RA, SLE, T1D, VIT	11
<i>ATXN2</i> : Involved in EGFR trafficking.			
<i>ZFP36L1</i> : Most likely functions in regulating the response to growth factors.	14q24.1	Cro, IBD, JIA, MS, PBC, RA, SLE, T1D	12
<i>CLK3</i> : Regulates the intranuclear distribution of splicing factors.	15q24.1-24.2	SLE	12
<i>CSK</i> : Non-receptor tyrosine-protein kinase that plays an important role in the regulation of cell growth, differentiation, migration and immune response.			
<i>CIITA</i> : Transcriptional regulator of HLA class II genes.	16p13.13	AA, Cro, IBD, JIA, MS, PBC, PS, RA, SLE, T1D	10
<i>SOCS1</i> : Cytokine signalling, tumour suppressor.			
<i>PRM1</i> , 2: Germ cell development.			
<i>PTPN2</i> : T cell differentiation and activation.	18p11.21	Cro, IBD, JIA, RA, T1D, UC	106
<i>ZNF335</i> : Gene expression and chromatin remodelling.	20q13.12	Cro, IBD, MS, RA	101
<i>UBASH3A</i> : Signal transduction in T cells and platelets.	21q22.3	AS, Cro, RA, T1D, UC	10
<i>ICOSLG</i> : B and T cell immunity.			
<i>RUNX1</i> : Development of haematopoietic stem cells.	21q22.12	JIA, RA	13
<i>UBE2L3</i> : Protein ubiquitination. May play a role in myelopoiesis.	22q11.21-11.22	Cro, JIA, MS, PS, RA, SLE, UC	12
<i>YDJC</i> : Probably catalyses the deacetylation of acetylated carbohydrates; an important step in the degradation of oligosaccharides.			
<i>HCFC1</i> : Transcriptional regulator of cell cycle progression.	Xq28	RA, SLE, T1D	12
<i>TMEM187</i> : Unknown function.			
<i>IRAK1</i> : Involved in Toll-like receptor (TLR) and IL-1R signalling pathways.			

Abbreviations: AA, alopecia areata; AS, ankylosing spondylitis; ATD, autoimmune thyroid disease; Cro, Crohn's disease; IBD, inflammatory bowel disease; JIA, juvenile idiopathic arthritis; MS, multiple sclerosis; PBC primary biliary cirrhosis; PS, psoriasis; PSC, primary sclerosing cholangitis; RA, rheumatoid arthritis; SJO, Sjogren's syndrome; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; UC, ulcerative colitis; VIT, vitiligo.
 Colour denotes: Candidate genes associated with T cell or B cell immunity (Blue); Innate immunity (green); myeloid lineage and antigen presentation (red); cytokine and/or chemokine activity (orange).

prevalence.³ There is a modest gender bias favouring females. The clinical effects of CD are broad and include gastrointestinal upset, chronic fatigue, nutrient deficiencies, other AID, osteoporosis, liver disease, infertility, sepsis and lymphoproliferative malignancy.¹ Diagnosis rests on demonstrating the characteristic intestinal damage of villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis.¹ Circulating antibodies to TG2, endomysium (which contains the target antigen TG2) and deamidated gliadin peptides (DGP) are highly sensitive for CD and are useful screening tests in the clinic, but the broad presentation of CD means detection rates remain suboptimal.⁴

Treatment of CD is strict and lifelong removal of the offending antigen, a gluten-free diet (GFD). Gluten describes the storage proteins (prolamins) from wheat, barley and rye. In some countries such as Australia and New Zealand, oats are also excluded from a GFD as they contain gluten-like proteins that are immunogenic in some CD patients.⁵ A strict GFD resolves symptoms and normalises CD-specific antibodies and enteropathy. However, maintaining satisfactory GFD compliance is challenging and many patients fail to achieve full disease remission. Therefore, the development of more effective treatment and diagnostic approaches are major research goals.

GENETIC INSIGHTS HIGHLIGHT THE CENTRAL ROLE OF THE IMMUNE SYSTEM

CD is a highly heritable polygenic disease with the strongest genetic risk linked to the HLA region.^{6,7} The specific HLA susceptibility genes encode for HLA-DQ2.5 (DQA1*05 and DQB1*02), HLA-DQ8 (DQA1*03 and DQB1*03:02) and HLA-DQ2.2 (DQA1*02:01 and DQB1*02:02).⁸ The DQA1 and DQB1 alleles encode the DQ

$\alpha\beta$ -heterodimer protein that resides on antigen-presenting cells (APCs) and facilitates gluten peptide and CD4⁺ T-cell interaction. In a large European study, 99.6% of 1008 patients with CD expressed HLA-DQ2.5, HLA-DQ8, HLA-DQ2.2 or HLA-DQ7 (DQA1*05+, DQB1*02 –).⁸ This contrasts with a prevalence of these haplotypes of ~30–50% in the general Western population.⁴

The high concordance rate for monozygotic twins (~80%) compared with the HLA-identical siblings (~30%) and dizygotic twins (~10%) underscores the importance of non-HLA genes in CD risk.⁹ More than 70 candidate genes in 42 non-HLA loci have been implicated in CD heritability based on genome-wide association studies^{6,10} and follow-up studies using ImmunoChip fine mapping or targeted analysis of disease-associated loci^{11–13} (Table 1). The individual contribution from each of these regions is small (odds ratio <1.5) and collectively they account for ~15% of the additional disease risk.¹⁴ Despite the small contribution, these loci highlight the importance of several immune pathways in CD pathogenesis, including roles for T- and B-cell activation, chemokine receptor activity and cell migration, cytokine binding, thymic differentiation of CD4⁺ and CD8⁺ T cells, stress pathways and innate immunity (Table 1). Underscoring the systemic nature of immune dysregulation in CD, only one gene has been shown to be relatively gut specific (*RGS1*).¹¹ *RGS1* expression is specific to the intestinal IEL compartment, and is believed to regulate chemokine receptor signalling and B-cell activation, and have a pivotal role in cell trafficking and tissue immunopathology (see below for the role of IELs in the intestinal lesion of CD). Most of the CD risk loci are shared in common with AID, such as rheumatoid arthritis (RA),

multiple sclerosis (MS) and type 1 diabetes (Table 1).^{10,12,15} However, natural killer (NK)-cell activation and interferon (IFN)- γ -production gene pathways appear to be selectively enriched in CD.¹⁶ Abadie *et al.*¹⁶ showed that the network of interactions between CD susceptibility genes can generate a range of functional outcomes, such as high IL-15 and/or IFN- α levels, with the specific combination of susceptibility genes potentially contributing to the heterogeneity of CD phenotypes.

Interestingly, ~90% of the identified risk loci map to non-coding regions (promoter regions, enhancers or non-coding RNA genes). This suggests that it is regulation of gene expression rather than changes at the protein-coding level that is more important for CD susceptibility and development.¹⁴ Recently, *lnc13* was identified as a long non-coding RNA harbouring six CD-associated single-nucleotide polymorphisms.¹⁷ Notably, *lnc13* regulates the expression of CD-associated inflammatory genes and its dysregulation may contribute to intestinal inflammation and autoimmunity.

Testing for HLA-DQ2.5/8/2.2 in the clinic is now common because it is an effective tool to exclude CD when these genotypes are absent.¹⁸ However, the test has limited positive predictive value for CD given the high prevalence of these genes in the general population. Tools that incorporate non-HLA single-nucleotide polymorphisms associated with CD may improve risk stratification and could also provide prognostic information.^{7,19,20} In collaboration with Inouye and colleagues, we showed that a genomic tool that generates a weighted score based on the presence of ~200 CD-associated single-nucleotide polymorphisms identified in previous studies^{6,10,12} provides a more fine-scaled estimation of CD risk than HLA typing,⁷ and better predicts CD development in first-degree relatives.²⁰ More studies are needed to establish the clinical utility and role for genomic testing.

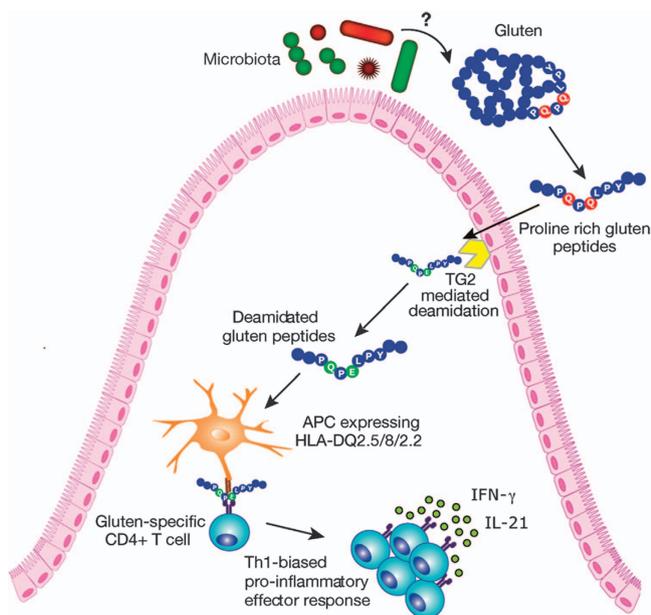


Figure 1 Key features of the CD4⁺ T-cell response to gluten. Gluten peptides containing T-cell epitopes resist gastrointestinal degradation due to their high proline content. Tissue TG2 catalyses the deamidation of gluten peptides, which can then bind more efficiently to the disease-relevant HLA-DQ molecules on APCs. Activated gluten-specific T cells secrete a variety of pro-inflammatory cytokines such as IFN- γ and IL-21 that contribute to the intestinal lesion (Figure 3). The microbiota may have several effects, including modifying gluten proteolysis and the net production of immunogenic peptides.

HLA AND THE GLUTEN-SPECIFIC CD4⁺ T-CELL RESPONSE

The strong HLA class II association in CD underscores the critical role of CD4⁺ T cells. Gluten-specific CD4⁺ T cells can be readily isolated and cloned from the intestinal lesion in CD and react to gluten peptides in an HLA-restricted manner. The gluten proteome is large and complex but the repertoire of gluten peptides that drive disease is highly restricted because of the specific binding requirements dictated by HLA-DQ2.5, DQ8 and DQ2.2. Indeed, there are more than 16 000 12mer gluten peptides from wheat, barley and rye that could potentially activate T cells²¹ but only just over 30 gluten-specific T-cell epitopes have been identified.²²

The gluten peptides harbouring T-cell epitopes are high in proline, rendering them resistant to gastrointestinal proteolysis as humans lack endogenous prolyl endopeptidases. A 33mer that contains six overlapping copies of immunodominant T-cell epitopes from wheat α -gliadin (the alcohol soluble fraction of wheat gluten) is particularly rich in proline, resistant to intestinal degradation and highly immunogenic in HLA-DQ2.5⁺ CD.²³ Although proposed as a major driver of wheat gluten toxicity, other important epitopes from protease resistant regions of ω -gliadin also elicit strong responses in most CD patients.²¹

Gluten-specific T cells preferentially respond to post-translationally modified gluten.²⁴ This modification, deamidation, is mediated by the ubiquitous enzyme TG2 (Figure 1). TG2 has a role in wound repair, is abundant in the small intestine and its activity is increased by tissue stress and inflammation.²⁵ TG2 is considered the autoantigen of CD as it is the target of IgA autoantibodies²⁶ and TG2 antibodies may have direct pathogenic effects (see below). TG2 has a critical role in CD pathogenesis by catalysing the conversion of glutamine (Q) to the more negatively charged glutamate (E). The specificity of TG2 is determined by the amino acids that flank glutamine residues, with preferential deamidation of glutamine in peptides conforming to the sequence QXP or QXXP (X denotes any amino acid).²⁷ This site selectivity is crucial as the negative charge introduced by glutamate serves as an anchor in the pocket of the peptide-binding groove of the HLA molecule. The greater stability of the major histocompatibility complex (MHC):peptide complex means that deamidated gluten peptides are more immunogenic. Post-translational modification is an important feature of AID such as RA, where both B- and T-cell responses are directed to specific citrullinated proteins introduced by the action of peptidylarginine deiminase enzymes. Although genetic variants in loci encoding these enzymes predispose to RA, a genetic link between TG2 and CD risk has not been reported.

Fine mapping of HLA-DQ2.5-restricted gluten T-cell epitopes has identified a preference for glutamate at anchor positions P4 or P6, and occasionally P7, corresponding to glutamine residues susceptible to deamidation by TG2.^{27–30} Most T-cell reactivity in HLA-DQ2.5-associated CD is directed toward the immunodominant wheat gluten T-cell epitopes DQ2.5-glia- α 1a (PFPQPELPY) and DQ2.5-glia- α 2 (PQPELPYPQ) contained within an α -gliadin peptide.^{28,31} These epitopes are dependent on the deamidation of a single glutamine to glutamate (represented by E) at P4 or P6 for optimal T-cell reactivity. Most CD patients mount responses to α -gliadin and ω -gliadin epitopes but responses to γ -gliadins are less frequent.²¹ Thus, the effect of TG2 is to generate more immunogenic T-cell epitopes, and through its site-selective targeting of gluten peptides, shape the immunogenic T-cell repertoire. Structural studies have provided additional insight into the gluten-HLA-T-cell receptor (TCR) complex and their interactions, explaining how deamidation enhances binding, and providing a structural basis for biased TCR gene usage.^{32,33}

In HLA-DQ8 CD binding favours glutamate at P1 and P9 to generate a more stable peptide:MHC complex.³⁴

The importance of generating an activation threshold in the peptide:MHC complex is exemplified by the gene-dose effect in HLA-DQ2.5 CD. Gluten presented by HLA-DQ2.5 homozygous APCs can induce at least a fourfold higher T-cell response than HLA-DQ2.5 heterozygous APCs due to the higher number of DQ2.5 $\alpha\beta$ -heterodimers available to the present gluten.³⁵ This appears to have clinical consequences, as HLA-DQ2.5 homozygous patients have the highest risk for CD development and a more severe phenotype demonstrated by earlier disease onset, greater villous atrophy and clinical features at presentation, and a slower rate of villous healing on a GFD.³⁶ Thus, genetic factors clearly have a role in the clinical expression of disease.

ACCESS TO GLUTEN-SPECIFIC T CELLS *IN VIVO* HAS ADVANCED T-CELL EPITOPE DISCOVERY

A major aim of AID research is to characterise the causative antigen driving the auto-reactive T-cell response but realising this goal has remained elusive. In CD, the relative ease in accessing small intestinal tissue by endoscopy has facilitated the isolation and cloning of gluten-specific T cells from intestinal biopsies. T-cell clones and lines are a well established and useful tool to identify T-cell epitopes, however the use of long-term culture with potent mitogens could change the composition of the T-cell population of interest and potentially favour the expansion of subpopulations that are uninformative.³⁷ Furthermore, this approach requires a costly and invasive procedure and has limited throughput. A complementary method to comprehensively define disease-specific T-cell epitopes relevant *in vivo* was needed.

This was realised when Anderson *et al.*³¹ showed that a 3-day oral gluten challenge could induce gluten-specific CD4⁺ T cells in the peripheral blood of treated CD patients, but not healthy controls, six days after commencing the challenge (Figure 2). These T cells are disease HLA-restricted, express the gut-homing $\beta 7$ integrin indicating an intestinal origin, and mostly express the CD45RO⁺ memory phenotype.^{37,38} By greatly expanding a memory-recall population *in vivo*, it became possible to directly sample antigen-specific CD4⁺ T cells from peripheral blood using sensitive functional assays such as

IFN- γ ELISpot,^{21,31,37-39} IFN- γ /IP-10 ELISA⁴⁰ or with tetramers.^{37,41} This means long-term *in vitro* culture can be avoided and endoscopy is not required. The ability to remove dietary gluten and reintroduce it (that is, oral gluten challenge) to provide a source of polyclonal gluten-specific T cells *in vivo* is unique to CD and has enabled unprecedented characterisation of the antigen-specific T-cell response.

Use of tetramers has comparable sensitivity to IFN- γ ELISpot and confirms the massive expansion of gluten-specific CD4⁺ T cells induced by gluten challenge, with T cells reacting to the DQ2.5-glia- $\alpha 1$ and DQ2.5-glia- $\alpha 2$ epitopes ranging from 1:1000 to 1:5000 among CD4⁺ T cells, equivalent to 1:5000 to 1:25 000 among peripheral blood mononuclear cells.³⁷ Using magnetic bead enrichment with tetramers, it is possible to detect gluten-specific T cells in the blood of treated and untreated CD patients without the need to expand this population by gluten challenge.⁴² Interestingly, 3-day gluten challenge concomitantly mobilises CD8⁺ and $\gamma\delta$ T cells into peripheral blood.⁴³ These T cells express intestinal homing markers and have a memory or effector phenotype, but their precise role and specificity for gluten is not defined.

The ability to readily isolate gluten-specific T cells has afforded an opportunity to assess the TCR repertoire expressed by T cells specific for the immunodominant α - and ω -gliadin epitopes. These studies highlight a biased and public TCR repertoire, with preferential TCR alpha and beta variable (TRAV and TRBV, respectively) gene use shaped by the specific epitope, specifically TRAV26/TRBV7-2 (DQ2.5-glia- $\alpha 2$),^{33,44-46} TRAV26/TRBV9 (DQ8-glia- $\alpha 1$)^{47,48} and TRAV4/TRBV4 (DQ2.5-glia- $\omega 2$).⁴⁹ There is a high level of binding affinity of TCRs for their cognate ligand, greater than that reported in more traditional autoimmune reactions. A non-germline encoded arginine residue in the CDR3 region is found in most TCRs specific for DQ2.5-glia- $\alpha 2$ and DQ8-glia- $\alpha 1$.^{45,48} This conserved residue appears to be a critical element in TCR recognition by interacting with the deamidated epitope. This suggests that TCR selection is shaped *in vivo* by a post-translationally modified antigen based on the structural requirements for binding.

We exploited the oral gluten challenge approach to undertake comprehensive T-cell epitope mapping studies in HLA-DQ2.5⁺ CD using gluten peptide libraries spanning the wheat, barley, rye and oat

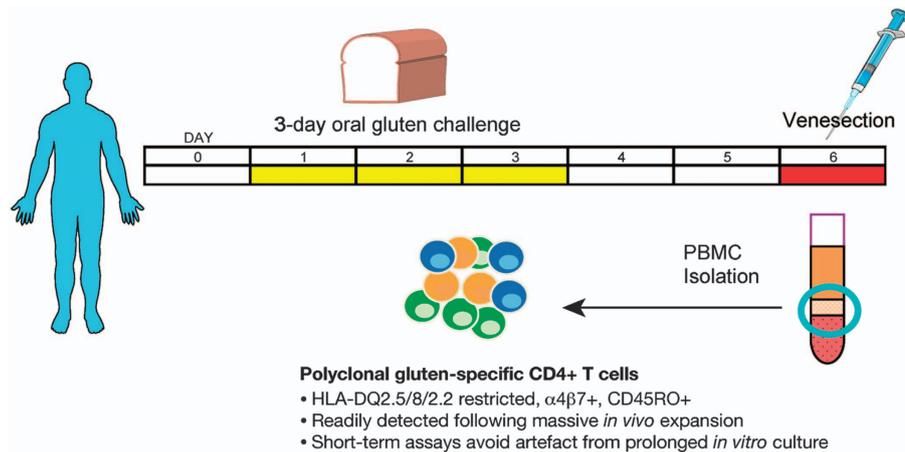


Figure 2 Exploiting oral gluten challenge to elicit disease-specific T cells *in vivo*. Short-term oral gluten challenge in treated CD mobilises a polyclonal gluten-specific CD4⁺ T-cell population *in vivo*. Patients with CD consume a gluten-containing cereal for 3 days, and blood is collected on day 6 to isolate peripheral blood mononuclear cells. By greatly expanding the gluten-specific T-cell population *in vivo*, a range of functional and immunophenotyping approaches can be readily employed without the need for prolonged *in vitro* expansion. Overnight functional assays such as IFN- γ ELISpot can be used for high throughput T-cell epitope mapping. Single-cell sorting by flow cytometry can be applied to the antigen-specific CD4⁺ T cells identified by proliferation or IFN- γ secretion, and these cells can be analysed for TCR repertoire or cultured to generate T-cell clones.

gluten proteome.^{5,21} The hierarchy of immunogenic peptides was highly consistent between CD patients with the same HLA type and was dependent on the cereal consumed. Three distinct peptides, encompassing the DQ2.5-glia- α 1a/ α 2, DQ2.5-glia- ω 1/ ω 2 and DQ2.5-hor-3 epitopes, accounted for most of the T-cell response to whole deamidated gluten. Although α -gliadin epitopes have been regarded as the most important driver of CD, this work showed these are only relevant after wheat is consumed and not after barley or rye. In fact, it is the ω -gliadin epitopes that are immunodominant after consumption of any of these cereals.²¹ Despite sequence similarity of the α - and ω -gliadin epitopes, their TCR repertoires are unique and show biased use of different TRAV and TRBV genes.⁴⁹ Appreciating the uniqueness of these distinct immunodominant epitopes has been crucial for the development of peptide-specific applications, including a novel CD diagnostic based on detection of whole blood IFN- γ release after gluten challenge⁴⁰ and a tolerogenic therapeutic vaccine (Nexvax2).⁵⁰

Employing this unbiased approach to T-cell epitope characterisation allowed us to confirm that the specificity and hierarchy of the T-cell response to dominant T-cell epitopes after wheat ingestion in children is comparable to that in adults with CD.⁴⁶ This suggests that by the time CD is diagnosed, irrespective of the age of the patient, the T-cell epitopes that drive disease are well established, subsequently remain stable over time and significant epitope spreading does not occur.

CD4⁺ T CELLS AND CD8⁺ IELS ARE REQUIRED FOR VILLOUS ATROPHY

The intestinal lesion in untreated (active) CD is characterised by infiltration with gluten-specific CD4⁺ T cells exhibiting a Th1-biased phenotype, with release of pro-inflammatory cytokines dominated by IFN- γ (Figure 3).⁵¹ IL-21 and IL-15 are also increased and drive development of the intestinal lesion in part by promoting activation of

cytotoxic CD8⁺ IELs. IL-21 regulates both adaptive and innate responses, drives production of IFN- γ , stimulates B-cell responses, upregulates the cytotoxic activity of IELs and renders effector T cells resistant to regulatory T-cell (Treg) suppression.⁵² IL-21 also supports development of Th17 cells, which are involved in the pathogenesis of a number of AID, but their relevance in CD is unclear. High IL-17 production from gluten-specific CD4⁺ T cells in active CD mucosa has been demonstrated^{53,54} but others have not been able to replicate this.⁵¹ IL-15 drives IEL cytotoxicity and loss of tolerance (discussed below) and can also promote the transformation of IELs into a T-cell lymphoma, a rare but highly fatal malignant complication of CD.⁵⁵

Protease resistant gluten peptides transit across the epithelium via transcellular and paracellular pathways.⁵⁶ Increased epithelial permeability may be mediated by the tight-junction disruptor zonulin.⁵⁷ Gluten peptides are deamidated and activate gluten-specific T cells, most likely within the lamina propria (Figure 1). Dendritic cells (DCs) are presumed to have a major role in presentation of these peptides to CD4⁺ T cells, however less is known about their identity and the location of this activation. After a 3-day gluten challenge there is an infiltrate of CD14⁺CD11c⁺ DCs in the intestinal mucosa and a reduction of CD11c⁺CD103⁺ DCs possibly due to migration to draining lymph nodes.⁵⁸ It is possible that both of these DC populations are involved in gluten presentation, to intestinal resident T cells, such as effector memory cells, or central memory T cells in the secondary lymphoid organs.

Activated gluten-specific CD4⁺ T cells are implicated in mediating intestinal damage but the relevant effector pathways for a long time remained unclear. Mediators upregulated by IFN- γ such as the matrix metalloproteinases,⁵⁹ which influence tissue remodelling, appear important but alone do not seem to fully account for the extent of enteropathy. Elevated numbers of CD8⁺ TCR $\alpha\beta$ ⁺ IELs are observed in the active CD lesion but until recently their significance was unclear. We now appreciate they play a key effector role in tissue destruction after they expand and adopt an NK cell-like phenotype (Figure 3). Distinct to the CD4⁺ T-cell response to gluten, this process is triggered by recognition of altered 'self tissue (epithelial stress) rather than a specific antigen.

In the healthy state, IELs express the inhibitory receptor CD94/NKG2A, but in active CD they express high levels of the activating NKG2D and CD94/NKG2C receptors.^{60,61} At the same time, 'stressed' intestinal epithelial cells in active CD patients express high levels of the stress-inducible MHC class I chain-related protein (MIC) molecules and non-classical MHC class I molecule HLA-E, which are the main ligands for NKG2D and CD94/NKG2C, respectively (Figure 3).^{61,62} IL-15 plays a key role by upregulating the activating NKG2D receptor and acting as a co-stimulatory molecule, the effect being to license cytotoxic IELs with the ability to kill intestinal epithelial cells expressing the stress-induced MIC molecules.

The causes of epithelial stress are not well defined. Gluten may trigger an innate response, however, the specific peptides and pattern recognition receptors have not been defined. An innate immune stimulatory effect of a gliadin sequence (A-gliadin p31-43) has been reported but this work has not been replicated.⁶³ Natural pest resistance proteins in wheat, the amylase trypsin inhibitors, have the capacity to bind TLR4 and stimulate an innate immune response in the intestine but their role in human disease is unclear.⁶⁴ Other drivers could include microbes, which might explain the epidemiological observation that gastrointestinal infection, such as rotavirus, is associated with risk for CD development and can precede disease onset.⁶⁵

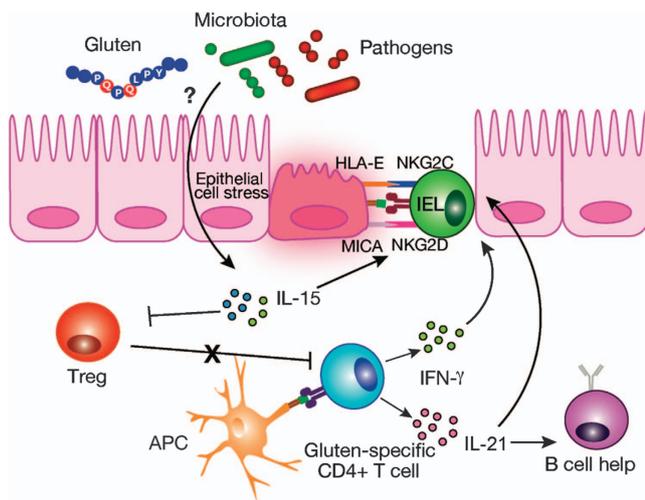


Figure 3 Cytotoxic IELs mediate killing of stressed intestinal enterocytes. The induction of an NK cell-like phenotype in CD8⁺ IELs mediates cytotoxic killing of stressed intestinal enterocytes. Stress-inducible molecules such as MIC and HLA-E are the ligands for the activating NKG2D and CD94/NKG2C receptors that, under the influence of IL-15, are highly expressed on IELs in active disease. Only intestinal enterocytes expressing IL-15 and the ligands for activating NK receptors will be killed. The drivers of epithelial stress may include gluten or inflammatory stimuli such as gastrointestinal infections. Gluten-specific CD4⁺ T cells produce IFN- γ and IL-21 that promote activation of IELs and stimulate B-cell responses. IL-15 renders effector T cells resistant to the suppressive effects of Tregs and, in the lamina propria, endows mucosal DCs with inflammatory properties promoting pro-inflammatory responses and preventing Treg differentiation.

Jabri and colleagues⁶⁶ offer an elegant model to explain how adaptive gluten immunity acts in synergy with epithelial stress to drive villous damage. They showed that some family members of CD patients with negative CD serology and normal small intestinal histology have stressed epithelium as demonstrated by ultrastructural alterations and elevated expression of stress markers and IL-15. However, the expression of high levels of inhibitory NK-cell receptors on cytotoxic IELs appears to prevent extensive epithelial cell killing. In contrast, patients with latent (potential) CD who have positive CD serology, that is, evidence of adaptive immunity to gluten, but normal small intestine, exhibit no epithelial stress response and activating NK-cell receptors are not upregulated. Consistent with a 'two-hit' model, the development of coeliac disease appears to depend on an adaptive immune response to gluten acting in synergy with epithelial stress to drive cytotoxic IELs and villous atrophy.

EMERGING INSIGHTS INTO THE B-CELL RESPONSE TO TRANSGLUTAMINASE AND GLUTEN

The contribution of B cells and TG2- and DGP-specific antibodies to CD pathogenesis has received less scrutiny than the role of CD4⁺ gluten-specific T cells. Anti-TG2 antibodies have a variety of *in vitro* effects including inhibiting intestinal epithelial cell proliferation, activating monocytes and inhibiting angiogenesis.⁶⁷ Interestingly, anti-TG2 IgA deposits are detectable in intestinal tissue before the development of overt CD, suggesting antibody production occurs early in disease alongside the gluten-specific T-cell response.⁶⁸ Deposits are also found in extra-intestinal sites such as muscle, liver, kidney and brain showing that TG2 is widely accessible to gut-derived circulating autoantibodies and may be responsible for some clinical manifestations in CD.⁶⁹ For example, known pregnancy complications in CD such as infertility, early pregnancy loss and intra-uterine growth retardation may be caused by impaired endometrial angiogenesis and placental damage mediated by anti-TG2 antibodies.⁷⁰ More research is needed to define the precise role of anti-TG2 antibodies in contributing to the enteropathy and extra-intestinal manifestations of CD.

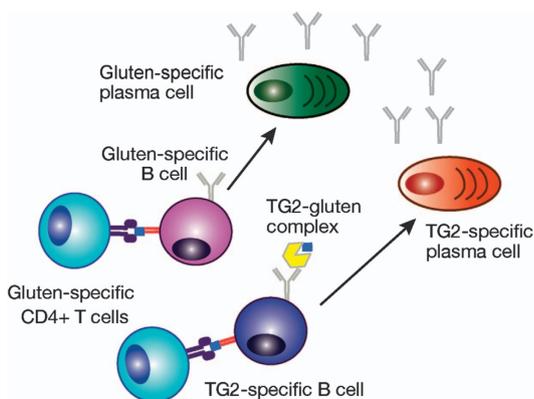


Figure 4 CD4⁺ T-cell and B-cell interaction may amplify the immune response to gluten and generate circulating antibodies. Circulating TG2- and DGP-specific antibodies are a feature of active CD and disappear when dietary gluten is excluded. Their occurrence requires the presence of CD-associated HLA types implicating a key role for T cells in their formation. The proposed model is that TG2-specific B cells internalise TG2-gluten complexes and then present them to gluten-specific CD4⁺ T cells, which in turn, provide help for antibody production. Gluten-specific B cells may receive help from gluten-specific CD4⁺ T cells if the B-cell epitope is linked to a T-cell epitope. B cells may enhance presentation of gluten peptides to CD4⁺ T cells to amplify the pro-inflammatory response to gluten.

The recent work of Sollid and colleagues has shed substantial light on TG2 and gluten-specific antibody production in CD. They showed a massive expansion of plasma cells targeting TG2 and gluten in the intestine of patients with active CD, with 5–25% of all IgA plasma cells specific for TG2.^{71,72} A GFD induces a substantial reduction in intestinal plasma cells and normalises plasma TG2 and DGP levels often within months, suggesting that long-lived plasma cells are not formed. Interestingly, they showed that antibody responses to gluten⁷³ and TG2⁷⁴ show restricted use of variable heavy (V_H) and variable light (V_L) chain gene segments with few somatic hypermutations despite high binding affinity. The low level of mutations could suggest that B-cell activation occurs extrafollicularly and is non T-cell dependent. The lack of an exceptionally long-lived plasma cell response would support this notion as these cells are generally formed in germinal centres in a T-cell dependent manner. Further work is required to understand the significance of these findings.

The co-occurrence of autoantibodies and HLA-restricted self-reactive T cells is common in AID and these components often localise together in affected tissue, for example, type 1 diabetes and thyroiditis. Close interactions between CD4⁺ T cells and B cells may be important for amplifying the inflammatory response, as B cells can present antigen to T cells and they in turn can provide help for autoantibody production (Figure 4). In CD, T-cell dependent antibody production is supported by the observation that TG2 and DGP antibody formation is strictly dependent on the presence of CD-associated HLA types as well as gluten.

As TG2-reactive CD4⁺ T cells have not been identified, it is proposed that TG2-specific B cells internalise TG2-gluten complexes and then present them to gluten-specific CD4⁺ T cells, which then provide help for antibody production⁷⁵ (Figure 4). T cell help may be important for loss of tolerance to TG2. As B-cell engagement will promote internalisation and efficient presentation at very low antigen concentrations, the result is a powerful amplification loop where B cells act as highly efficient APCs enhancing the gluten-specific CD4⁺ T-cell response. Antigen-presenting B cells could potentially modify the T-cell epitope repertoire displayed to T cells and influence functional responses. Further insight is needed into how TG2- and gluten-specific B cells are activated, where this occurs and how gluten-specific T and B cells interact *in vivo*.

In contrast to the detailed knowledge of T-cell epitopes in gluten, mapping of gluten-specific B-cell epitopes is restricted to wheat gliadin. Several linear epitopes have been defined in α- and γ-gliadin by screening phage-display and synthetic peptides in adult CD patients,⁷⁶ and a peptide microarray approach has defined several discontinuous gliadin epitopes.⁷⁷ Notably, several B-cell epitopes lie within or are in close proximity to immunodominant T-cell epitopes, for example, the B-cell epitope QPQQPF in γ-gliadin is encompassed within DQ2.5-glia-ω1/2.⁷⁸ This raises the possibility that antibodies could influence T-cell recognition of the related epitope and modify gluten-specific responses *in vivo*.

WHY AND HOW DOES GLUTEN TOLERANCE FAIL?

The Western diet contains a large amount of gluten (~15–20 g day⁻¹) and HLA genetic susceptibility to CD is seen in almost half the population. Why only 1–2% of the population develop CD and the remainder do not is of substantial interest. Importantly, in HLA-DQ2.5⁺ volunteers without CD, T-cells targeting immunodominant gluten epitopes (DQ2.5-glia-α1a and -α2, DQ2.5-glia-ω1 and -ω2 and DQ2.5-glia-γ2) are not present in intestinal biopsies and few are present in the peripheral blood.⁷⁹ The tetramer-binding T cells show lower gluten-specific proliferation, do not express gut-homing

markers, do not display a Treg phenotype and do not have a biased TCR repertoire typical of responses to certain gluten epitopes in CD patients. Thus, healthy individuals with HLA susceptibility to CD generally do not mount pro-inflammatory or regulatory T-cell responses to the dominant gluten epitopes that cause CD.

The situation in CD is quite different. Perhaps counter-intuitively, high levels of induced Foxp3- IL-10-producing type 1 (Tr1) Tregs and naturally occurring Foxp3⁺ Tregs are present in the CD intestinal lesion and peripheral blood, with increased levels associated with active disease.^{80–82} The antigen specificity of these reported Tregs is unknown. Accumulation of Foxp3⁺ Tregs in target organ tissue is noted in AID such as RA and inflammatory bowel disease and may be due to attraction of Tregs to the inflamed tissue in a non-antigen-specific manner. Gianfrani *et al.*⁸³ showed that IL-10-producing gliadin-specific Tr1 clones expanded from treated CD intestine are able to inhibit the proliferation of gliadin-specific CD4⁺ effector T-cell clones. Furthermore, IL-10 and TGF-β can be detected in high amounts in the CD intestinal lesion.^{84,85}

Failure of the suppressive capacity of Tregs in CD could be one explanation for broken tolerance, and although some studies have supported this concept,^{86,87} others have shown function is retained, at least *in vitro*.^{82,88} These conflicting results highlight the difficulty in identifying Treg populations that have true suppressive capacity *in vivo*. As commonly employed Treg markers such as CD25 and Foxp3 are not solely expressed on Tregs and can be upregulated in activated T cells, identifying more specific markers is of interest. Reduced IL-7 receptor (CD127) expression can identify CD4⁺ T cells that have a suppressive Foxp3⁺ phenotype even when CD25 is low or absent,⁸⁹ but this is yet to be examined in detail in CD. CD134 (OX40) has been used as a marker for antigen-specific T cells and CD39 can be used to identify antigen-specific Tregs.^{90,91} This approach has been used to show CD39⁺ Tregs compose a substantial proportion of T cells induced by 3-day oral wheat ingestion in CD, and they remain functionally suppressive (Cook *et al.*, in submission). The ability to effectively monitor antigen-specific Tregs will provide a powerful research tool and may be useful in assessing tolerogenic strategies for CD in clinical trials.

IL-15 PROMOTES LOSS OF TOLERANCE TO GLUTEN

As the defect in gluten tolerance in CD is not readily explained by a defect in the number or recruitment of Tregs, attention has turned to IL-15. This pleiotropic cytokine can block the ability of TGF-β to suppress activation of mucosal T cells⁸⁴ and impair the ability of CD25⁺ Foxp3⁺ Tregs from the blood and intestinal biopsies of CD patients to suppress effector CD4⁺ T cells *in vitro*.⁸⁸ In an HLA-DQ8 mouse model overexpressing IL-15 in the lamina propria, IL-15 in combination with retinoic acid altered the tolerogenic phenotype of intestinal DCs and promoted a pro-inflammatory response characterised by the release of IL-12 and IL-23.⁹² Along with retinoic acid, these cytokines then promote Th1 and Th17 polarisation, and the failure of induction of Foxp3⁺ Tregs to dietary gliadin. The authors note that although retinoic acid is considered important in the generation of Tregs and oral tolerance, its adjuvant effect is consistent with its usage as a pro-inflammatory adjuvant in anti-tumour immunity. Notably, anti-TG2 IgG and IgA antibodies are generated in the transgenic mice but no enteropathy develops, supporting the concept that in the absence of the epithelial stress and increased IL-15, adaptive gluten immunity alone is insufficient to induce tissue damage.

THE EMERGING LINK BETWEEN ENVIRONMENT, THE MICROBIOME AND GLUTEN IMMUNITY

Environmental factors contribute to the development of CD in genetically susceptible individuals but what they are, and how they do this, is poorly understood. Population studies associate several factors with increased CD risk such as perinatal and childhood infections, use of antibiotics and proton-pump inhibitors, higher socioeconomic status and better hygiene, and elective Caesarean section.³ Delayed introduction of dietary gluten may reduce the time of onset of development CD but it remains unclear if there is an optimal time for gluten introduction.^{93,94} These controlled studies failed to demonstrate a protective role for breast feeding in reducing CD risk. Many of the implicated environmental factors may act by altering the composition of the microbiome.⁹⁵

Dysbiosis or perturbations in the composition of resident commensal microbial communities away from those found in healthy individuals, are reported in a range of autoimmune and inflammatory diseases, including CD. The dysbiosis is characterised by an abundance of Proteobacteria and a decrease in *Lactobacillus*.⁹⁵ Epidemiological data support an association between dysbiosis and increased risk of CD but there is little understanding of how it might influence gluten-specific immunity *in vivo*. *In vitro* data supports a broad range of influences of microbes on immune responses to gluten.⁹⁵ This includes roles in modifying Treg induction, epithelial cell stress and IEL activation, phenotypic and functional maturation of DCs and pro-inflammatory cytokine production, intestinal permeability, and the induction of CD4⁺ T-cell responses.⁹⁵ There is no convincing evidence of a role for molecular mimicry in CD, and no microbe-specific T cells cross-reactive with gluten have been identified. Verdu and colleagues⁹⁶ recently showed that gluten metabolism in the small intestine of gnotobiotic mice is differentially affected by opportunistic pathogens such as *Pseudomonas aeruginosa* (*Psa*) and commensal bacteria such as *Lactobacillus* spp. We showed that the peptides generated from the proteolytic action of these bacteria show strikingly different levels of immunogenicity to gluten-specific T cells induced in CD patients after 3-day wheat challenge *in vivo*, with *Psa* increasing and *Lactobacillus* spp. reducing the load of immunogenic peptides.⁹⁶ An imbalance of *Psa* over *Lactobacillus* spp. *in vivo* could favour the net generation of immunogenic gluten peptides and promote disease (Figure 1). Microbe–dietary–host interactions may be an important modulator of autoimmune risk in genetically susceptible people. Targeting this interaction may provide novel approaches to reduce disease risk or modify the activity of established disease.

TRANSLATIONAL SIGNIFICANCE OF UNDERSTANDING COELIAC DISEASE PATHOGENESIS

A range of experimental therapies for patients with CD are in development.⁵⁰ Some aim to reduce the load of immunogenic gluten peptides contacting the mucosal immune system by enzymatically degrading gluten, sequestering it or reducing its passage across the mucosa. These quantitative approaches may reduce the adverse effects of small amounts of dietary gluten but would not replace the need for this treatment. Blockade of IL-15 may be useful in refractory CD, where IL-15 drives inflammation despite adequate gluten removal. Pre-clinical approaches to selectively inhibit TG2 or block HLA are also being explored.⁵⁰

Restoring tolerance to the causative antigen in AID is highly attractive but complicated by the need to sufficiently characterise the driving antigen and monitor auto-reactive T cells.⁹⁷ In contrast to the more ‘classical’ AID, development of epitope-specific approaches in

CD are feasible because the causative T-cell epitopes in gluten are well characterised.²¹ The therapeutic vaccine Nexvax2 that targets the T-cell epitopes relevant in HLA DQ2.5⁺ CD is entering Phase 2 clinical trials to assess efficacy.⁵⁰ Induction of tolerance may be achieved through several mechanisms, such as deletion of naive T cells specific for the dominant peptides composing the vaccine or the induction of Treg differentiation.⁹⁷ Tregs may inhibit pro-inflammatory T cells and exert linked suppression by blocking the differentiation of naive T cells that recognise self antigens released as a result of tissue damage (epitope spreading). They may also act directly at the level of memory T cells to promote anergy and cause deletion.⁹⁷ Pharmacogenetics will be important in the selection of peptides relevant to the CD HLA-DQ type. Modifying gluten immunity using the hookworm *Necator americanus* has shown some promise and larger controlled studies are underway.⁹⁸

CONCLUSION

Our view of CD has evolved from a gastrointestinal malabsorptive illness to an immune disease characterised by HLA-dependence, gluten-specific CD4⁺ T cells, autoantibodies to TG2 and systemic clinical manifestations. CD exemplifies how an illness can have autoimmune-like features without having to be driven by an endogenous antigen. Indeed, CD challenges many traditional notions of what might be reasonably considered an 'autoimmune disease'. For example, formation of autoantibodies occurs to a self-antigen despite the driver being an exogenous dietary protein. The tissue destruction of CD is mediated by cytotoxic IELs directed against 'altered-self' in a highly targeted manner, as only the enterocytes expressing IL-15 and the stress-induced ligands for NK receptors will be killed. On this basis, Jabri and Sollid² argue that IELs in CD could be regarded as self-reactive. If one is to accept this designation, CD could be reasonably considered as a model of organ-specific autoimmunity.

So how well does CD fulfil the definition of an AID? The modified Witebsky's postulates employ three-tiers of evidence to establish defining criteria for AID.⁹⁹ This includes evidence that is direct, for example, transfer of disease by pathogenic autoantibody; indirect, for example, identifying autoantibodies or T cells within the target lesion or reproduction of disease in experimental animals; or circumstantial, for example, clinical clues, such as clustering of AID within the same patient. In practice, most AID, including those regarded as 'bona fide' AID, do not fulfil all of the criteria. It is notable that CD fulfils several, the seeming distinction being the requirement for an exogenous driving antigen. However, Jabri and Sollid² raise an interesting point—could exogenous drivers be important in the pathogenesis of more traditional AID? Exploring the role of non-self antigens as drivers in other AID may be informative. As in the case for CD, this may be via a mechanism distinct from molecular mimicry.

Although diagnosis of CD still rests on demonstrating intestinal villous atrophy,¹ this may underestimate the true burden of disease. Some patients with positive CD serology and a CD-susceptible HLA type but minimal enteropathy suffer gluten-induced symptoms and morbidity, and clinically benefit from a GFD.¹⁰⁰ This supports the notion that CD is a systemic illness, the hallmark of which is aberrant immunity to gluten, and that gastrointestinal damage is an important but not necessarily exclusive manifestation. In the future it will be important to determine if other approaches, such as determining the presence of a pathogenic gluten-specific T-cell response, might have greater clinical utility and accuracy in diagnosing CD.

The rise in CD prevalence makes approaches to improve diagnosis and enhance treatment outcomes a pressing clinical need. Several novel therapies for CD are in development, and their success will test

the accuracy of our knowledge of CD pathogenesis. Uncovering how environmental factors and the microbiome shape the balance between gluten tolerance and immunity in genetically susceptible individuals may reveal entirely new approaches for disease prevention and management.

CONFLICT OF INTEREST

MYH and JAT-D are co-inventors of patents pertaining to the use of gluten peptides in therapeutics, diagnostics and non-toxic gluten in coeliac disease. JAT-D is a shareholder of Nexpep Pty. Ltd and a consultant to ImmusanT Inc. Nexpep Pty. Ltd and ImmusanT Inc. were formed to develop novel diagnostics and treatments for coeliac disease.

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