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Triggers and drivers of autoimmunity: lessons from coeliac disease

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Preface

Coeliac disease, an inflammatory disease of the small intestine, shares key features with autoimmune disorders, such as susceptibility genes, presence of autoantibodies and T cell-mediated destruction of specific cells. Strikingly, however, continuous exposure to the exogenous dietary antigen gluten and gluten-specific adaptive immunity are required to maintain immunopathology. These observations challenge the notion that autoimmunity requires adaptive immune activation towards self-antigens. Using coeliac disease as an example, we propose that other exogenous factors might be identified as drivers of autoimmune processes, in particular when evidence for T cells with specificity for self-antigens is lacking.

Immune processes that lead to tissue destruction and disease need to be initiated and then maintained. Thus, conceivably, there are separate triggers and drivers of these processes. A trigger can be defined as an event that activates the disease process in some way, whereas a driver can be defined as a factor that powers and maintains the disease. We generally assume that in an organ-specific autoimmune disorder, the triggers are exogenous factors that break self-tolerance by induction of innate immune responses and activation of dendritic cells, whereas drivers are self-antigens that activate adaptive immunity. When exogenous factors are thought to drive the adaptive immune response it is in the context of molecular mimicry, where T cells that recognise the exogenous antigen crossreact with self-antigens that are the real drivers of the disease. Thus, exogenous antigens are not typically considered to be drivers of autoimmune disorders.

Coeliac disease, which has key features of autoimmune disorders, challenges this view. It was first described by the Greek physician Aretaeus, who lived around the first century AD, as an intestinal disorder associated with malabsorption and diarrhoea. In the 1940s, the Dutch paediatrician Willem-Karel Dicke discovered that coeliac disease is caused by the consumption of cereal gluten proteins^{1, 2} (Box 1). Later, it was found that the finger-like projections of the small bowel mucosa — the intestinal villi — are absent in patients with coeliac disease who consume gluten³. This explains why patients can suffer from malabsorption. The condition is frequent with a prevalence of about 1:100, and it occurs selectively in individuals expressing HLA-DQ2 or HLA-DQ8 (REF⁴). The presence of highly disease specific transglutaminase 2 (TG2)-specific autoantibodies⁵ allows the diagnosis of the disease. Thus, similar to patients with organ-specific autoimmune disorders, patients with coeliac disease have autoantibodies and suffer from the destruction of a specific tissue cell type by CD8⁺ T cells. Yet we also know that these autoimmune features

require the presence of gluten and that HLA-DQ2- or HLA-DQ8-restricted gluten-specific CD4⁺ T-cell responses have a central role in disease pathogenesis (Figure 1). We therefore propose that on the basis of coeliac disease, we should consider the possibility that exogenous antigens may drive autoimmune disorders.

Box 1

Gluten proteins and coeliac disease

Gluten is the collective name for the storage proteins found in grains of wheat, barley and rye¹⁰⁰. The name gluten comes from the cohesive and elastic ball of proteins which remains after kneading and washing wheat flour in water to remove the starch. Grain proteins in wheat, barley and rye are very similar, and although the gluten ball only can be formed from flour of wheat, the term gluten is often used to name all these types of protein. Typically, gluten proteins are rich in glutamine and proline residues. The high content of proline makes them resistant to gastrointestinal digestion. In wheat, gluten proteins are divided into gliadins and glutenins, whereas the gluten proteins of barley and rye are termed hordeins and secalins, respectively. Patients with coeliac disease raise CD4⁺ T cell responses to several distinct gluten peptides, and these peptides are recognised in the context of coeliac disease-associated HLA-DQ molecules³⁸. In addition, the patients make antibodies specific for gluten proteins.

In the first part of this Opinion, we discuss the key genetic and immunological features of coeliac disease from the perspective of an unknown driver. In the second part of the article, we dissect how gluten and the gluten-reactive CD4⁺ T cell response drive the autoimmune processes that are characteristic of coeliac disease. In the last part, we contrast the roles of drivers and triggers in the pathogenesis of coeliac disease and discuss the relevance of these concepts for understanding autoimmunity in general.

Autoimmune features of coeliac disease

Considering the key genetic and immunological features of coeliac disease, it becomes apparent that if we did not know that coeliac disease relates to gluten consumption, we would classify it as a typical organ-specific autoimmune disorder targeting the small intestine.

Genetics

Similar to other autoimmune diseases, coeliac disease is a polygenic disorder for which the MHC locus is the single most important genetic factor. The MHC locus accounts for 40–50% of the genetic variance in the disease. Coeliac disease has a very strong HLA association. The great majority of patients carry a particular variant of HLA-DQ2 (DQA1*05:01, DQB1*02:01; also known as DQ2.5). Those who are not DQ2.5⁺ are almost all HLA-DQ8⁺ (DQA1*03, DQB1*03:02) or they carry another variant of HLA-DQ2 (DQA1*02:01, DQB1*02:02; also known as DQ2.2) (see REF⁶ for further details). As all coeliac disease patients carry particular HLA variants, HLA can be considered a necessary, but not sufficient, factor for disease development. HLA testing is much used in the clinic to exclude the diagnosis of coeliac disease.

Several non-MHC genes have also recently been discovered as susceptibility factors in coeliac disease. So far, 39 loci with 57 independent association signals have been described⁷. Many of these loci harbour genes related to immunity, particularly to B cell and T cell function. Together these loci have been estimated to account for 14% of the genetic variance of the disease⁷. Interestingly, many of the susceptibility loci for coeliac disease are

shared with those for autoimmune diseases such as type-1 diabetes and rheumatoid arthritis^{8–12}. Typically, disease-associated single-nucleotide polymorphisms (SNPs) locate to regulatory DNA marked by DNase I hypersensitive sites (DHSs)¹³. The SNPs often affect the recognition of gene enhancers by transcription factors, thereby resulting in differences in gene expression. The implicated transcription factors are a part of regulatory networks that seem to be shared between coeliac disease and other autoimmune diseases¹³. For example, of the SNPs associated with autoimmune disorders that fall within DHSs, a quarter localise to recognition sequences of transcription factors that interact with IRF9, which indicates that JAK–STAT-mediated type-I interferon (IFN) responses might have important roles in mediating diverse autoimmune disorders. The sharing of genetic loci between coeliac disease and other autoimmune diseases suggests that there are shared immunological mechanisms and shared autoimmune features.

Autoantibodies

Patients with active coeliac disease produce autoantibodies, mainly specific for TG2 (REF⁵), and assessment of such autoantibodies has an increasingly important role in clinical practice (Box 2). The production of autoantibodies is a typical feature of many autoimmune diseases, such as anti-neutrophil cytoplasmic antibodies (ANCA) in vasculitis¹⁴ and anti-citrulline protein antibodies (ACPA) in rheumatoid arthritis¹⁵. TG2 is an enzyme with many functions, one of which involves modifying gluten peptides as a substrate (Box 3). In coeliac disease, antibodies specific for TG2 are produced locally in the mucosa¹⁶, and possibly elsewhere. Recently, plasma cells producing TG2-specific antibodies were visualised in coeliac disease lesions and isolated by use of labelled TG2 antigen¹⁷. On average, 10% of the plasma cells of a disease lesion are TG2 specific, and most produce IgA. The TG2-specific plasma cells disappear when the patients commence a gluten-free diet. Monoclonal antibodies generated from single plasma cells recognise conformational epitopes of gluten and display restricted VH and VL usage and few somatic hypermutations¹⁷. This could suggest that the antibodies are generated without the involvement of T cells. However, the strict occurrence of TG2-specific antibodies in persons with certain HLA types¹⁸ and the fact that the avidity of the antibodies decreases by reverting them to presumed germline configuration¹⁷, indicate that such antibodies have undergone affinity maturation and that their development is T cell dependent.

Box 2

Autoantibodies and coeliac disease

Autoantibodies in coeliac disease were initially detected as reticulin-specific antibodies by staining of rat tissue^{101, 102}. Subsequently IgA endomysium-specific antibodies (EMA), detected by staining of monkey oesophagus¹⁰³ or human umbilical cord¹⁰⁴, were described. The main antigen recognised by reticulin-specific antibodies and EMA was identified as the enzyme transglutaminase 2 (TG2)⁵. Autoantibodies specific for calreticulin¹⁰⁵ and actin¹⁰⁶ are also associated with active coeliac disease, but in contrast to TG2-specific antibodies these are not used in routine diagnostics. TG2-specific antibodies are found in serum as IgA, IgG and IgM isotypes. Assaying for TG2-specific IgA is most commonly used in clinical practice as this test has the highest disease specificity and sensitivity¹⁰⁷. In fact, the disease specificity and sensitivity for this test is higher than for any other autoantigen–disease association. Previously, detection of histological changes in small intestinal biopsies, involving blunting of intestinal villi and infiltration of inflammatory cells, were considered mandatory for the diagnosis of coeliac disease. Biopsy examination is no longer mandatory in the diagnostic work-up of

children¹⁰⁸, mainly due to the very strong diagnostic performance of the autoantibody tests.

Box 3

Biology of transglutaminase 2 (TG2)

TG2 is a ubiquitously expressed enzyme with multiple functions¹⁰⁹. It is found intracellularly bound to GTP and extracellularly bound to Ca²⁺. The Ca²⁺-associated form can modify specific glutamine residues in polypeptides by either crosslinking them with primary amines — and when this is a lysine residue forming protein crosslinks — or converting the glutamine residue to glutamate by reaction with water in a process termed deamidation¹⁰⁹. Many gluten peptides are excellent substrates for TG2 leading to posttranslational modification¹¹⁰. The TG2 enzyme is inactivated by oxidation and can be made active again under reducing conditions¹¹¹. The enzyme is not constitutively active *in vivo*⁷⁰. The mechanisms underlying its activation are not fully identified, but evidence suggests that inflammation, tissue destruction and a reductive environment are associated with enzyme activity.

The strict correlation between HLA type and presence of autoantibodies is also observed in several other autoimmune disorders. In rheumatoid arthritis, there is a strong association between the HLA-DRB1 shared epitope and ACPA¹⁹. Recently, it was reported that ANCA-associated vasculitis consists of two genetically distinct subsets: anti-myeloperoxidase ANCA is primarily associated with HLA-DQ polymorphisms, whereas proteinase 3 ANCA is primarily associated with HLA-DP polymorphisms¹⁴. These HLA associations with distinct autoantibodies suggest that there are specific T cells that control autoantibody formation.

Leukocyte infiltration

Autoimmune lesions are often characterised by the accumulation of leukocytes. Likewise, coeliac disease lesions show infiltration of leukocytes, both in the epithelium and in the lamina propria²⁰. In the epithelium, the infiltration of CD8⁺ T cells, termed intraepithelial cytotoxic lymphocytes (IE-CTLs), dominates. In the lamina propria, there is increased density of many cell types including plasma cells, CD4⁺ T cells and antigen presenting cells.

Tissue destruction

Similar to type-1 diabetes, in which β -islet cells of the pancreas are the only cell type affected, coeliac disease is characterised by the selective destruction of intestinal epithelial cells. The basal membrane of the epithelium remains intact and there are no tissue ulcerations²¹. The targeting of a specific cell type in coeliac disease implies that specific receptor–ligand interactions are involved. In coeliac disease, intestinal epithelial cells or enterocytes express non-classical MHC class I molecules that are recognised by activating natural killer (NK) cell receptors, which are upregulated on IE-CTLs^{22–24}. The two most highly expressed NK cell receptors are NKG2D and CD94–NKG2C, which recognise MIC and HLA-E molecules induced on epithelial cells of patients with coeliac disease^{24, 25}. NKG2D and MIC (and related molecules) have also been implicated in the pathogenesis of rheumatoid arthritis and type-1 diabetes^{26, 27}. Furthermore, IL-15 — a cytokine that is upregulated in tissue cells targeted during the autoimmune process^{28–30} — is increased in the epithelium of patients with coeliac disease³¹. It functions in a cell contact-dependent manner to increase NKG2D expression on IE-CTLs and is a co-stimulatory molecule for the T cell receptor (TCR) and NK cell receptors^{23, 32}. Together, the alterations observed at the

level of intestinal epithelial cells and IE-CTLs in coeliac disease lead to dysregulated activation of IE-CTLs and villous atrophy. The two non-mutually exclusive mechanisms proposed for intestinal epithelial cell destruction in coeliac disease are direct NK-cell receptor-mediated destruction based on the recognition of inducible non-classical MHC molecules and potentially — as a result of a decrease in the TCR activation threshold^{33, 34} — TCR-mediated killing through the recognition of low avidity (self) antigens. In both cases, epithelial cells are destroyed by IE-CTLs with autoreactive properties that recognise self or modified-self antigens through activating NK cell receptors and/or the TCR⁴.

Thus coeliac disease has many of the features that classify disorders as autoimmune (Figure 2). Looking at the research frontiers of other autoimmune diseases, primary research objectives in coeliac disease would likely be to identify TG2 reactive T cells and to understand why T cell tolerance to TG2 is broken. This is not so, and coeliac disease does often not figure on lists of autoimmune conditions. The reason is that it is perceived as a food hypersensitivity disorder where gluten is the culprit food antigen. And in one way this is true; gluten is the driver of the condition.

Gluten: the driver of coeliac disease

The gut lesion of most patients with coeliac disease completely normalises when gluten is excluded from the diet, and it reappears when the patients again eat gluten. Both the presence of autoantibodies specific for TG2 and the increased number of IE-CTLs are gluten dependent as they change upon gluten elimination/challenge^{35, 36} (Figure 1). Patients with coeliac disease, but not healthy controls, have HLA-DQ2- or HLA-DQ8-restricted CD4⁺ T cells in the gut mucosa that are reactive to gluten³⁷. Given the strong HLA association, CD4⁺ T cells are probably key players in the development of the disease.

HLA-DQ-restricted anti-gluten CD4⁺ T cell response

Gluten-reactive T cells are present in the lamina propria of patients with coeliac disease and preferentially recognise deamidated gluten peptides³⁸ in the context of disease-associated HLA molecules, but not other HLA molecules^{39, 40} (Figure 3). In these deamidated peptides, certain glutamine residues have been converted to glutamate by the enzymatic action of TG2^{41, 42}. T cell presentation of negatively charged peptides may explain the association with HLA-DQ2 and HLA-DQ8 molecules, as both these MHC molecules have preference for binding peptides with negatively charged anchor residues⁴³. Not only T cells, but also antibodies, of patients with coeliac disease preferentially recognise deamidated gluten peptides⁴⁴. This situation, of having antibodies specific for both the enzyme that mediates a posttranslational modification and the modified product, is paralleled in rheumatoid arthritis. Patients with rheumatoid arthritis have antibodies specific for citrullinated antigens (ACPA) as well as for the peptidylarginine deiminase (PAD) enzyme that mediates the citrullination (reviewed in REF¹⁵). This may suggest shared mechanisms for antibody formation in the two diseases.

The presence of a large number of proline residues renders gluten resistant to digestion by intestinal enzymes. The generation of long peptides and deamidation provide an explanation as to why immunogenic peptides of gluten are generated⁴⁵. Importantly, analysis of the cytokine profile of gluten-specific T cells has shown that they produce IFN- γ (T_H1 immunity) and IL-21, but not IL-17 (REF⁴⁶). Multiple types of immune reaction are probably required to create the lesion, but the gluten-reactive CD4⁺ T cells can be considered a gatekeeper explaining that MHC is a necessary but not sufficient genetic factor^{47, 48} (Figure 3). As we discuss in the following, there is evidence that the autoimmune processes in coeliac disease can be driven by the activation of gluten-specific CD4⁺ T cells.

TG2-specific autoantibodies

The strict dependence on gluten exposure for the production of TG2-specific antibodies in coeliac disease³⁵ suggests that gluten is implicated in driving the formation of these antibodies (Figure 3). A possible mechanism in keeping with this notion could be that gluten-reactive CD4⁺ T cells provide the required help to TG2-specific B cells in a hapten-carrier-like manner by involvement of TG2-gluten complexes^{49, 50}. Gluten peptides form covalent complexes when incubated with TG2, either as a thioesterbond to the active site cysteine residue of TG2 (enzyme-substrate intermediate) or as an isopeptide bond linkage to lysine residues of TG2 (REF⁵¹). When TG2-specific B cells bind and internalise such complexes through their surface immunoglobulin, deamidated gluten peptides can be released by proteolytic cleavage or upon cleavage of the isopeptide-thioester bonds in endosomes; deamidated gluten peptides can then bind HLA-DQ2 or HLA-DQ8 for presentation to gluten-reactive T cells. Some experimental support for this model has been obtained¹⁷. Further, assays measuring antibodies reactive with deamidated gliadin peptides are better predictors of disease than assays measuring antibodies reactive with native gliadin peptides⁵², which suggests that B cells specific for the posttranslationally deamidated peptides may receive help from the same gluten specific T cells. Of note, TG2 specific as well as gluten specific B cells can serve as antigen presenting cells for gluten specific T cells, and in this way serve as important amplifiers of the CD4⁺ T cell response to gluten.

IE-CTL activation

The proliferation and activation of IE-CTLs requires the presence of gluten and parallels the activation of gluten-specific CD4⁺ T cells (Figure 3). When patients start a gluten-free diet, the number of IE-CTLs usually returns to a normal frequency, expression of NK-cell receptors on IE-CTLs decreases and expression of non-classical MHC class I molecules on epithelial cells is downregulated^{22–24}. We have proposed that the licensing of IE-CTLs to kill requires two independent but interrelated alterations in the intestinal mucosa: activated gluten-specific CD4⁺ T cells and a stressed epithelium expressing high levels of IL-15 and non-classical MHC class I molecules^{25, 53}. It remains to be determined how epithelial stress, adaptive anti-gluten T-cell immunity, B cell immunity and activation of TG2 might interplay to determine the acquisition of a killer phenotype by IE-CTLs.

Triggers of coeliac disease

As to the concept of triggers and drivers, if gluten is the driver of coeliac disease, are there triggers implicated in coeliac disease? There is no experimental evidence to suggest that gluten could promote the activation of autoreactive T cells through molecular mimicry, as has been proposed for other triggers of autoimmunity^{54, 55} such as Cocksackie-virus and autoimmune myocarditis⁵⁶. Nonetheless, could there be other triggers that help to initiate the CD4⁺ T cell response to gluten and induce alterations in epithelial cells that contribute to the activation of IE-CTLs?

The need for a trigger

HLA is a necessary, but not sufficient, genetic factor in coeliac disease, suggesting that additional factors are required for disease development. In particular, the poor digestion of gluten peptides and their deamidation by TG2 provide an explanation as to why immunogenic gluten peptides of relative high affinity for HLA-DQ2 and HLA-DQ8 are generated, but this does not explain why inflammatory T cell responses instead of regulatory T cell responses are mounted against gluten in the intestinal environment, which normally promotes tolerogenic responses to oral protein antigens⁵⁷. In a mouse model overexpressing IL-15 in the lamina propria to levels similar to those found in patients with active coeliac disease, IL-15 in conjunction with retinoic acid was shown to disrupt oral tolerance and

induce the differentiation of inflammatory dendritic cells that produce IL-12p70 and promote the differentiation of IFN- γ -producing T cells (T_H1 immunity)⁵⁸. Type-I IFNs are also found to be upregulated in the intestinal mucosa of patients with active coeliac disease⁵⁹, although the increased levels appear to be moderate and lower than in skin lesions of lupus erythematosus⁶⁰. Type-I IFNs, through their ability to activate dendritic cells, promote T_H1 type immunity and induce loss of oral tolerance, could potentially be an alternative pathway causing loss of tolerance to gluten. Furthermore, the enzyme TG2 is not constitutively active *in vivo*, but inflammation is associated with enzyme activation (Box 3). Finally, another key aspect of coeliac disease is the upregulation of IL-15 and non-classical MHC class I molecules in the epithelium. It is likely that the CD4⁺ T cell response has a role, but is not sufficient alone to induce these alterations in the intestinal epithelium. Together, these observations are in line with the concept that a strong adaptive immune response is typically paired with an innate component that facilitates the adaptive response⁶¹. What might such innate factors be in coeliac disease (Figure 3)?

Gluten as a trigger as well as a driver?

The simplest model would be that gluten is also a trigger having innate properties. As discussed above, two mechanisms have been invoked to explain the induction of inflammatory T cell responses to gluten through the induction of dendritic cells with an inflammatory phenotype: the first one involves IL-15 upregulation in the lamina propria⁵⁸ and the second one may involve type-I IFN⁶² (Figure 3). Gluten has been shown to promote the upregulation of IL-15 (REF⁶³) and possibly IFN- γ in the lamina propria by unknown mechanisms. In addition to inducing innate cytokines that alter the tolerogenic phenotype of dendritic cells, studies suggest that gluten might have a role in the effector destructive phase of the disease also by activating epithelial cells^{63, 64}, and promoting the upregulation of IL-15 and non-classical MHC class I molecules on intestinal epithelial cells^{22, 65}. However, the literature on 'innate properties' of gluten is heterogeneous and conflicting, and there is no consensus on the mechanisms by which gluten could trigger innate immune responses (discussed in REF²⁵). Recently, it was demonstrated that innate stimulation by wheat proteins is not mediated by gluten proteins themselves, but rather by wheat amylase trypsin inhibitors, which co-purify with certain gluten proteins and which activate toll-like receptor 4 (REF⁶⁶). In addition, it is not clear why gluten might have innate effects only in certain individuals and what would determine it acquiring such properties in a given individual at a certain time. It is possible that this acquired 'sensitivity' to gluten requires the encounter between a predisposing genetic background and an environmental factor that renders host cells responsive to the innate properties of gluten. Thus, even supposing that gluten does have some 'innate properties', it would be difficult to explain the initiation and effector phase of the disease without the existence of triggers that would alter the microenvironment in such a way that gluten can induce activation of the mucosal immune system. Such triggers could be viruses and bacteria of the gut.

Viruses and type-I IFN

Viral infections, in particular enteroviral infections such as rotavirus infections^{67, 68}, have been suggested to increase the incidence of coeliac disease and other autoimmune disorders such as type-1 diabetes^{53, 69}. Furthermore, studies in mice using poly(I:C) as a mimic for double-stranded RNA virus infection indicate that viral infections may promote activation of TG2 (REF⁷⁰). Because viral infections lead to type-I IFN production^{53, 71} and type-I IFNs have immunostimulatory properties⁷², we assume that viral infections might trigger autoimmune disorders as a consequence of type-I IFN induction. Type-I IFNs could also be induced by bacterial infections^{73–76}, and potentially by as yet undefined host factors that are not related to infections. Future studies will establish the role that is played by type-I IFNs and other virus-induced innate factors in coeliac disease pathogenesis (Figure 3). We also

need to investigate how type-I IFNs could remain upregulated in the intestinal mucosa of patients with coeliac disease and how their production could become gluten dependent once viruses have been cleared. Unless chronic viral infections are involved, it would seem that viral infections might be responsible for triggering the initial type-I IFN induction, but that other as yet undefined factors are responsible for maintaining increased type-I IFN levels in the intestinal mucosa. How gluten could contribute to the upregulation of type-I IFN also remains to be determined.

Microbiota

Complex disorders result from the interaction between environmental factors, the microbiota, and the state of the host. The high increase in incidence of autoimmune disorders cannot be explained by genetic drift and is thought to be the result of changes in the environment. It is now recognised that the microbiota can affect on intestinal immune homeostasis and the health of epithelial cells^{77–79}. Short chain fatty acids produced by the microbiota promote epithelial cell proliferation through activation of G coupled protein receptors and might have an impact on the intestinal immune system through their histone deacetylase inhibitory properties. Furthermore, the intestinal microbiota comprises bacteria that promote regulatory immune responses (symbionts) and bacteria that promote inflammatory immune responses (pathobionts)^{77–79}. As for other autoimmune disorders, such as type-1 diabetes, multiple sclerosis and rheumatoid arthritis^{80, 81}, differences in the composition of bacterial communities have been reported for the microbiota of patients with active coeliac disease compared with healthy controls^{82–86}. Alterations were also reported for patients on a gluten-free diet^{82, 83, 85, 86}. In particular, decreases in butyrate-producing bacteria *Faecalibacterium prausnitzii* and representatives of the genus *Bifidobacterium* were reported in patients with coeliac disease^{87, 88}, as well as an expansion of potentially pathogenic bacteria such as *Escherichia coli* and *Staphylococcus* spp.^{89–91}. It remains to be determined whether these changes are a consequence of the presence of an inflammatory milieu or have an active role in causing disease (Figure 3). Additional studies are required to assess whether dysbiosis might constitute a trigger for loss of tolerance to gluten and full licensing of IE-CTLs.

Altogether, although there is strong support for the role of microorganisms as triggers of innate immune activation, there is no evidence of a role for molecular mimicry in coeliac disease. In other words, we do not have any evidence so far that coeliac disease pathogenesis is the result of a T cell response against a microbial peptide that is crossreactive with gluten. For example, it has been proposed that adenoviruses might contain sequences shared with gluten⁹², but the presence of T cells crossreactive with gluten and adenovirus could not be identified. Importantly, coeliac disease also illustrates that there is no need to invoke a role for molecular mimicry to explain loss of tolerance⁹³. However, although individual potential culprits have been identified, we still have a long way to go before demonstrating how a microorganism in the context of a particular genetic background might trigger disease through the induction of IL-15 or type-I IFN production.

Conclusions and perspective

Analysing the drivers of coeliac disease leads us to conclude that an exogenous antigen (gluten) can lead to the induction of autoimmune features such as autoantibodies and the destruction of a specific tissue type. We hence propose that the adaptive immune response in an autoimmune disease does not need to be directed at or even related to the antigens that are the target of the autoimmune process. Furthermore, analysis of coeliac disease pathogenesis also leads us to question the concept that destruction of a specific cell type requires cognate TCR–peptide MHC interactions. IE-CTLs destroy intestinal epithelial cells in coeliac disease based on the presence of stress signals rather than because they are

specific for an epithelial cell antigen. Only cells that upregulate IL-15 and non-classical MHC class I molecules will be targeted by IE-CTLs. The receptor–ligand interactions involve non-cognate recognition of low affinity self or microbial peptides by the TCR, and recognition of inducible non-classical MHC class I molecules by NK cell receptors. Thus, the presence in the tissue targeted by an autoimmune process of CD8⁺ T cells with lymphokine killer activity and/or a very low activation threshold could point towards an autoimmune process driven by an exogenous factor.

These observations lead us to question how much we know about the drivers of other autoimmune diseases? Can we eliminate with certainty that the driver is not, at least at some point, an exogenous factor, the role of which did not involve molecular mimicry? For example, it has been proposed that the driver of rheumatoid arthritis⁹⁴ and inflammatory bowel disease⁹⁵ might be a dysregulated immune response against bacteria that compose our microbiota. In accordance with the microbiota being a driver of disease, mouse models of colitis require the microbiota⁹⁶, and in humans antibiotic treatment⁹⁷ and diverting the faecal stream away from sites of disease⁹⁸ can reduce the activity of inflammatory bowel disease. Primary biliary cirrhosis is an interesting example where a bacteria, *Novosphingobium aromaticivorans*, that shares antigenic epitopes with the mitochondria is recognised by NKT cells and functions as the initial driver of the disease, before classical autoreactive T cells take over⁹⁹.

Thus there may be a spectrum of autoimmune disorders, with at one end diseases in which exogenous antigens are the only driver, in the middle diseases where exogenous antigens are the initial driver, and at the other end diseases where the immune response is selectively directed against self-antigens. We argue that when considering the pathogenesis of autoimmunity, thinking about drivers that might not be self-antigens could be highly informative and could change the way we diagnose, prevent and treat autoimmune disorders. The identification of exogenous drivers in autoimmunity is a difficult but not impossible task. Taking into account MHC association, posttranslational modifications, the antigen and the tissue, it is possible to design strategies that will facilitate the identification of potential drivers outside the human proteome.

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Box 4**Novel approaches to identify disease relevant antigenic epitopes:
examples from coeliac disease**

One approach relates to the identification of peptides that undergo posttranslational modification. — A highly complex proteolytic digest of gluten was incubated with TG2 and a biotinylated amine that is a TG2 substrate, and which allowed fishing and isolation of gluten peptides that are preferentially targeted by TG2 (REF ¹¹²). Strikingly, 75% of the identified peptides contained the intact or parts of known T-cell epitopes. This type of approach is relevant in diseases where enzymes undertake posttranslational modifications. A third approach, making use of the accumulation of disease-relevant plasma cells in the disease lesion as seen in coeliac disease, involves cloning monoclonal antibodies from single plasma cells by recombinant DNA techniques¹⁷. The T cell receptor recognises antigen as the complex of antigenic peptides bound to MHC molecules, whereas B cell receptors/antibodies recognise antigen directly and with higher affinity than the T cell receptor. Thus antibodies are better suited than T cell receptors to identify their target antigens. Care should be made to screen potential target antigens that are correctly folded also to capture antibodies recognising conformational determinants. For example, the epitopes of TG2 recognized by antibodies from coeliac disease patients are conformational¹⁷. A third approach relates to identification of antigenic peptides which bind to MHC molecules associated with disease, like HLA-DQ8 associated with type-1 diabetes and HLA-DR4 associated with rheumatoid arthritis. In coeliac disease this approach has been used to identify T cell epitopes of gluten which are presented by the HLA-DQ2.5 and HLA-DQ2.2 molecules (Dørum, Sollid et al., unpublished observations).

Figure 1. Autoimmune phenomena and adaptive anti-gluten immunity are associated with coeliac disease and are dependent on gluten exposure

Coeliac disease pathology is characterised by the presence of gluten-specific adaptive immunity and the presence of autoimmune phenomena such as cytotoxic autoantibodies and CD8⁺ T cells that mediate enterocyte destruction without being gluten specific. Autoimmune and non auto-immune phenomena, as well as tissue pathology, recede when dietary gluten is eliminated and reoccur when gluten is reintroduced.

Figure 2. Coeliac disease and autoimmune disorders share many common immune mechanisms MHC class II molecules, post-translational modifications (PTM), autoantibodies (auto-Ab), IL-15, IFN- α , T helper 1 (T_H1) type immunity, IL-21, natural killer cell receptors (NKR) and non-classical MHC class I molecules have been reported to be implicated in many autoimmune disorders.

Figure 3. Dietary antigen drives autoimmune processes in coeliac disease

Triggers such as viruses, pathogens and pathobionts activate antigen-presenting cells and epithelial cells. Antigen-presenting cells acquire proinflammatory properties and present gluten to induce the activation of gluten-specific HLA-DQ2- or HLA-DQ8-restricted CD4⁺ T cells. Because transglutaminase 2 (TG2) and gluten form complexes, TG2-specific autoreactive B cells internalize TG2–gluten complexes and present gluten peptides on HLA-DQ2 or HLA-DQ8 at their surface. Gluten-specific B cells can bind and present deamidated gluten peptides in a conventional manner. The gluten-specific CD4⁺ T cells provide help to both autoreactive TG2-specific and gluten-specific B cells, which will differentiate into antibody producing plasma cells. Activated gluten-specific CD4⁺ T cells also provide signals (that remain to be fully defined) to pre-activated epithelial cells, which upregulate IL-15 and non-classical MHC class I molecules. Consequently, intraepithelial cytotoxic T lymphocytes (IE-CTLs) acquire lymphokine activated killer activity and a decreased T cell receptor (TCR) activation threshold and will kill epithelial cells based on the recognition of stress signals. Whether IE-CTLs with a decreased TCR activation threshold will recognise low affinity epithelial antigens and antigens of the microbiota through their TCR remains to be determined. The autoimmune phenomena are boxed.