

# Pathogenetic sequences in coeliac disease: closing the jigsaw puzzle

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## Abstract

Coeliac disease is one of the best understood models of adaptive immunity in which a known dietary component triggers over-presentation of a known autoantigen in genetically-predisposed individuals. The dynamics of this gene-nutrient interaction and the mechanism responsible for accelerated programmed death of the enterocytes lining the upper small intestine are explored in order to generate insight into the large number of candidate pharmacological agents that may well aid or replace cumbersome dietary treatment in years to come.

## Dr. Dicke kicks off to a good start

Research into the cause of coeliac disease (CD) was put on the right path by Dr. William Karell Dicke, Director of the Childrens' Hospital in The Hague, Netherlands. Dicke was impressed in 1932 by reports of a child whose diarrhoea recurred on repeated challenge with bread and rusks. During the Dutch winter of starvation of World War II, cereal rationing serendipitously cured many of Dr. Dicke's coeliac patients. By 1953 Dicke<sup>1</sup> had published a paper with J. H. Van de Kamer, a nutritional biochemist showing that the alcohol-soluble (gliadin) fraction of the water-insoluble (gluten) component of wheat was responsible for steatorrhoea in CD. After some initial scepticism it became apparent that the flat appearance of duodenal biopsies, initially ascribed to a biopsy forceps-related crush artifact was the result of a gross immunological attack on the enterocytes lining the intestinal villi. For many years the exact autoantigen remained elusive.

## Key words

Coeliac disease, tissue transglutaminase, gluten, villous atrophy

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## Back to the drawing board

The earliest event in the pathogenesis of CD is now known to involve the binding of toxic but non immunogenic gliadin peptides on the enterocyte luminal membrane to the surface receptors of "zonulin", a novel signaling protein that induces polymerization of actin microfilaments in the enterocyte cytoskeleton.<sup>2</sup> These intracellular strands interact with the proteins of the intercellular "tight junctions" permitting paracellular passage of gliadin peptides into the lamina propria without subjecting them to the natural process of intracellular lysosomal degradation that would otherwise limit their antigenicity (Figure 1).

Certain peptides released by the luminal digestion of gluten are resistant to the usual further digestion by peptidases located in enterocyte brush borders. Some of these peptides (e.g. the poorly digested 33 amino acids peptide (56-89)) contain several immunostimulant sequences which may elicit a damaging immune response in the lamina propria.<sup>3</sup> Another possible scenario is that minor amounts of gluten peptides cross the enterocyte by specific transcytotic mechanisms where they may be partly digested by cathepsins to release immunogenic fragments (Figure 1).<sup>4</sup>

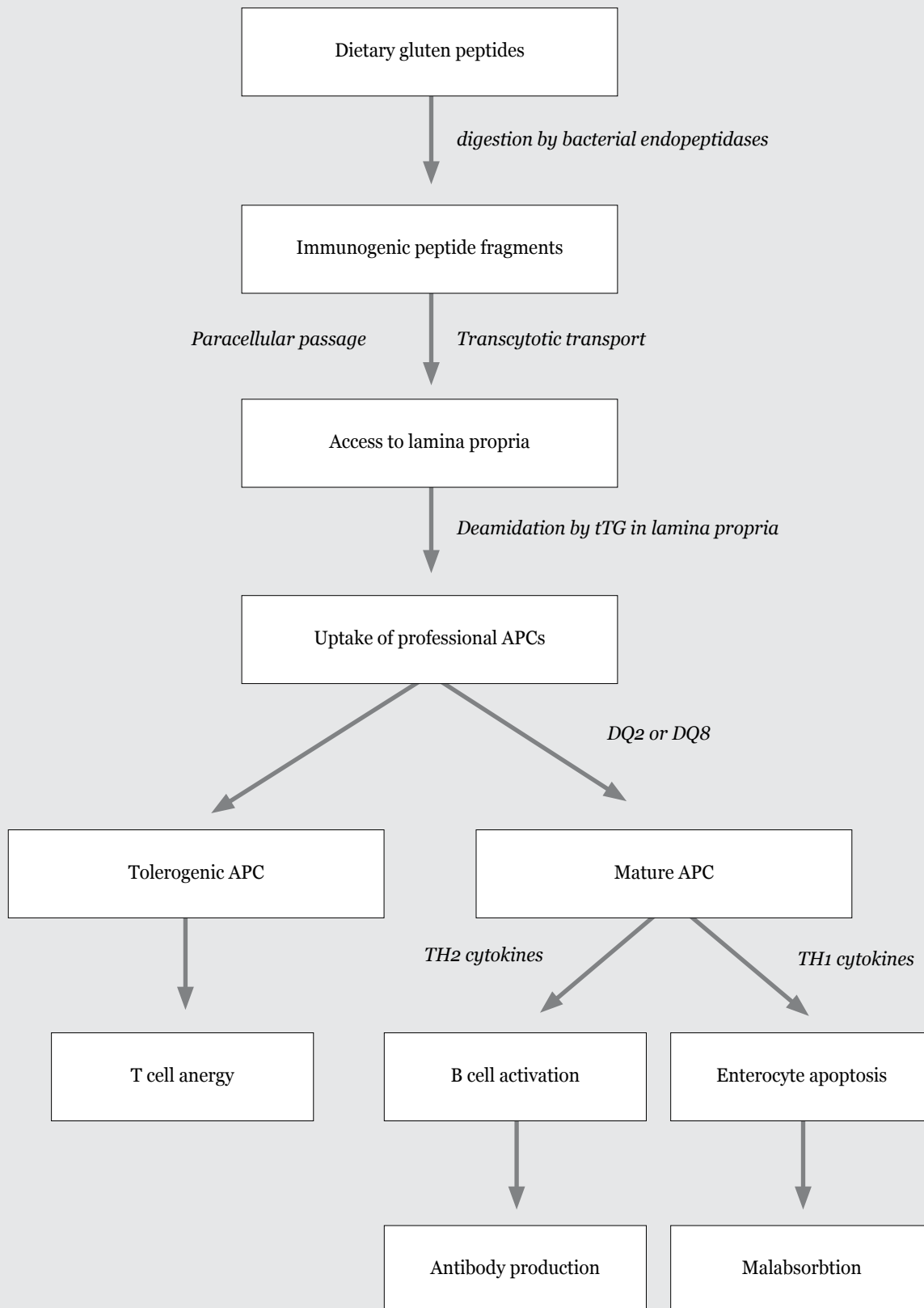
Whatever their mode of transport, experiments show that immunogenic peptides gain access to dendritic cells in the intestinal lamina propria. These professional antigen-presenting cells (APC) stimulate T-helper cells to induce apoptosis (programmed cell death) of enterocytes and also elicit a damaging humoral response. The race is now on to identify which dietary peptides are truly immunogenic in the hope of finding the epitopes which fit into the antigen binding groove of APCs. Various peptides, obtained by tryptic digestion of A-gliadin are now under intense scrutiny by food chemists.

## Genetically driven warfare: enter tissue transglutaminase

Coeliac disease is one of the best understood models of adaptive immunity against a known trigger that is determined by variability in primitive antigen presenting molecules (MHC glycoproteins) encoded by the HLA locus of the MHC genes on chromosome 6. Coeliac disease develops almost exclusively in patients who carry the HLA class II molecules DQ2 or DQ8.<sup>5</sup>

Tissue transglutaminase (tTG) has emerged as the culprit autoantigen responsible for chronicity in coeliac disease.<sup>6</sup> Antibodies against tTG can be detected by a number of

**Figure 1:** Sequence by which gluten peptides lead to intestinal epithelial damage  
(APC: antigen presenting cells)



**Table 1:***Glossary of immunological terms and abbreviations***APC**

Antigen presenting cells are bone marrow-derived cells that hold antigens for presentation to T lymphocytes.

**Apoptosis**

A genetically determined or triggered process of programmed cell death, or “cell suicide”; which removes harmful or aging cells from the body without tissue damage.

**CD4+**

A marker carried by helper T cells which help orchestrate the antibody response in contrast to the other cluster of differentiation (CD) markers on cytotoxic or suppressor T cells which are involved in cell-mediated immunity.

**HLA**

Human leucocyte antigens are genetically determined antigens on the surface of cell membranes that serve to identify a cell as self or nonself.

**IEL**

Intraepithelial lymphocytes are T lymphocytes that are situated among the enterocytes lining the intestinal villous.

**Immunodominant**

Possessing an area (called an epitope) on the surface of an antigen capable of stimulating a specific immune response.

**Motif**

A short, highly conserved, often repetitive, region in a protein sequence.

**MHC**

Complex of genes coding for a large family of cell-surface proteins that bind peptide fragments and present them to T-lymphocytes to induce an immune response. MHC class I molecules exist on all cells and hold and present foreign antigens to CD8 cytotoxic T lymphocytes if the cell is infected e.g. by a virus. MHC class II molecules are found on APCs and display antigen to activate CD4+ cells.

**TH1 response**

An acquired T cell response characterized by high cytotoxic T lymphocyte activity relative to the amount of antibody production. The TH1 response is promoted by CD4 “TH1” subset of T-helper cells.

**TH2 response**

An acquired T cell response characterized by high antibody production relative to the amount of cytotoxic T lymphocyte activity. The TH2 response is promoted by CD4 “TH2” T-helper cells.

commercially available serologic kits. tTG is found in the enterocyte brush border, fibroblasts and inflammatory cells of the lamina propria and beyond the intestine in skin, kidney and lung cells. tTG maintains the integrity of the healthy intestine by cross-linking several matrix proteins in the epithelial basement membrane. The cross-linking of glutaminase-containing sequences such as gliadin peptides with lysine-containing peptides which abound in matrix collagen may enhance the immunogenicity of gliadin by generating neo-epitopes which persist in the basement membrane.<sup>7</sup>

Inflammation and low pH favour deamidation of gliadin rather than its cross linking. Deamidation provides negatively charged peptides which have a higher affinity for the antigen binding site of HLA DQ2 and DQ8 molecules expressed on the surface of APCs such as the dendritic cells of the intestinal epithelium.<sup>8</sup> Dendritic cells internalize, partly degrade and selectively process gliadin peptide fragments before re-expressing them bound to the MHC group on their plasma membrane. Alfa-2 gliadin contains a 33 amino acids digestion resistant peptide containing three stretches of a conserved motif that is found in many immunodominant gluten peptides.

Dendritic cells then migrate through the lamina propria and mesenteric lymph node where they associate freely and promiscuously with CD4+ and T-Helper lymphocytes (TH). Meanwhile the gliadin derived peptides are internalized by dendritic cells into endosomes where they are partially digested. Only a few of the resulting peptide fragments will fit the antigen binding groove of MHC class II molecules synthesised in the endoplasmic reticulum of the APC and encoded by highly polymorphic HLA DQ2 and DQ8 genes which determine disease susceptibility. When MHC bound peptides on the surface on the APC are recognized by T cell receptors (TCR) on Class II restricted TH cells, the number of TH cells triggered will determine whether the reaction stops here (immunological tolerance) or progresses to cell-mediated immune damage.

Intercellular adhesion molecules such as ICAM1 help to approximate the antigen to the TCR but bound antigen will not trigger the T cell unless co-stimulatory molecules are present. Absence of costimulation is one of nature’s way of promoting immunological tolerance. After triggering, TH cells produce a predominantly TH1 response by secreting the cytokines IL2, TNF $\alpha$  and IFN $\gamma$ . These cytokines which specialize in cell-mediated cytotoxicity allow mesenchymal cells in the lamina propria to release destructive matrix metallo-proteinases (MMP) that greatly accelerate the apoptosis or programmed cell death of villous enterocytes. Other TH cells release TH2 cytokines that induce proliferative and humoral responses, stimulating B lymphocytes to produce antibodies against gluten and tTG that are detectable in the circulation (Figure 1).

**Enter the intra epithelial lymphocytes (IELs)**

Cytotoxicity in the lamina propria is complemented by a unique population of lymphocytes called intra-epithelial lymphocytes (IEL) that proliferate between the enterocytes on

their basolateral surface. IELs express the marker CD8 and are more likely to express the g/d TCR than CD4<sup>+</sup> T cells which usually carry the a/b TCR. IELs recognize a narrow spectrum of antigen in the context of an MHC class I molecule. Their function is to tackle and kill, via IFN $\gamma$  production and apoptosis any enterocytes that express abnormal cell proteins on their damaged surface. Their CD45RO phenotype indicates that they are also memory cells capable of rapid enterocyte destruction.<sup>9</sup> IELs are probably the progeny of MHC class I restricted T cells which were “educated” in the lymphoid tissue of the ileal Peyer’s patches by antigen-sampling “M cells” before circulation and homing to the enterocytes covering the intestinal villous.<sup>10</sup>

### **The murder weapons revealed**

Cytotoxic cells kill enterocytes in many ways. They may degranulate releasing serum proteases called “granzymes” and the subunits of membrane drilling proteins called “perforins”<sup>11</sup> which are endocytosed by enterocytes. Once inside their innocent victims perforins self-assemble releasing powerful granzymes into the cytoplasm. Further triggering of apoptosis results from IFN secretion and the expression of Fas ligand which binds to enterocyte membranes. This irreversible “death sequence” in doomed enterocytes usually starts with receptor-mediated elevations in intracellular calcium which activates destructive calcium-dependent enzymes such as caspases and endonucleases.

### **The battle ground from above: a histopathologist’s perspective**

The duodenum appears to take the brunt of the immune assault while the jejunum and ileum show less severe histological damage. This is fortuitous as endoscopic duodenal biopsies are sensitive markers of disease activity even though they do not indicate the extent of small bowel damage. Duodenal histology provides a mere single-frame glimpse of a process of accelerated enterocyte apoptosis. Marsh<sup>12</sup> had described four phases which are thought to occur consecutively. The earliest (Marsh I) lesion consisting of increased IELs (< 30 per 100 enterocytes) is seen in patients suffering from Dermatitis Herpetiformis. The base of each villous is surrounded by 4-10 crypts of Lieberkuhn which contain rapidly proliferating enterocyte progenitors. Crypt cells move 1-2 cell positions per hour in the enterocyte monolayer up the side of the villous reaching the villous tip in about 5 days. The mucosa responds to apoptosis by hyperplasia and branching of the crypts which compensate for accelerated shedding of enterocytes at the villous tips (Marsh II). Some patients will “decompensate” with partial (Marsh III) or complete (Marsh IV) villous atrophy. Tumour necrosis factor activated by TH1 cells triggers intestinal fibroblasts to release matrix metalloproteins which stimulate mesenchymal migration and proliferation increasing the bulk of the lamina propria and causing further deepening of the crypts and effacement of villi.

A Marsh III lesion with a crypt-to-villous height ratio above 1.0 is the gold standard for diagnosing coeliac disease. Biopsy

reports should be read in the context of serological tests since small intestinal bacterial overgrowth, giardiasis, and cow’s milk protein allergy can cause villous atrophy. Marsh I and II biopsies present a diagnostic dilemma and patients should be warned of this limitation before proceeding to endoscopic biopsy. Wahab *et al*<sup>13</sup> demonstrated progression to Marsh III lesions in only 12 of 38 patients with Marsh I biopsies after an 8 week gluten challenge. All 12 patients experienced symptom relief on a subsequent gluten free diet (GFD) but histological response was variable. Failure of symptoms to respond to a gluten challenge predicted absence of histological improvement on a GFD in many cases. Whether GFD prevents complications in histological non-responders is not known.

Many patients with latent CD exhibit a loss of the decrescendo pattern of IELs which are usually more crowded at the base of the villous that at its apex in normal individuals.<sup>14</sup> However up to 25% of patients with a relative increase of IELs in the villous distal sides and tips do not develop coeliac disease on follow up. The diagnostic value of this finding is therefore limited.

### **Digestion disarmed and dismantled**

The immune attack on enterocytes grossly reduces absorptive surface area and deprives the intestine of precious disaccharidases located in the microvillous brush border. Increased intestinal permeability makes the small intestine more susceptible to the osmotic pull of undigested disaccharides which manifests as reducing substances in the stool. Further osmotic diarrhoea results from unabsorbed fatty acids which also ferment in the colon to produce excess hydrogen and methane and volatile fatty acids causing a distended abdomen, explosive steatorrhea and perianal burning in children. Rarely a microscopic colitis and/or an autoimmune pancreatitis add to the digestive chaos.

### **Beyond the gut: the mysteries deepen**

Many extraintestinal manifestations of CD are poorly understood and probably harbour important clues as to its pathogenesis. CD osteopathy (osteomalacia + osteopenia) has been traditionally attributed to malabsorption of dietary calcium complicated by negative calcium balance and secondary hyperparathyroidism. tTG is also present in bone where it promotes the cross-linking of a non-collagenous bone protein substrate called osteocalcin greatly increasing its rate of incorporation into growing bone collagen. Immunofluorescent studies on sera of CD patients showed high titres of antibody to areas of active mineralization in foetal rat tibia.<sup>15</sup> An immune attack on tTG causing reduced collagen incorporation into growing bone would therefore also explain CD osteopathy.

tTG is also implicated in many cases of sporadic ataxia without features of multiple system atrophy i.e. autonomic and pyramidal features. These so called “gluten ataxias”, which often feature cerebellar atrophy on MRI and a silent sensory-motor axonal neuropathy on EMG often show deposition of IgA tTG

in the jejunum and brain blood vessels as well as high titres of circulating anti-gliadin antibodies. As in dermatitis herpetiformis where the immune attack is directed toward epidermal tTG, jejunal biopsies may be misleadingly normal.

The possible role of tTG in the causation of Alzheimer's disease (AD) and Huntington's chorea are also very intriguing. CSF tTG is raised in many AD patients and tTG is up-regulated in the AD brain and localises to the microtubule "tau" protein with its characteristic neurofibrillary tangles. It is not yet known whether excessive cross-linkage and consequent insolubility of tau may damage microtubule function by increasing its persistence.<sup>16</sup>

tTG is also elevated in the brain of many Huntington's Disease (HD) patients who exhibit characteristic neuronal intranuclear and cytosolic inclusion bodies containing the mutant "Huntington protein." Again polyamination and excessive cross-linking of truncated forms of this mutant protein are being scrutinized as putative mechanism for neural damage.<sup>17</sup>

## Conclusion

A better understanding of the way in which immunogenic peptides derived from gliadin are processed by enterocytes and modified by tissue transglutaminases in the intestine and other organs to enhance their presentation to the immune system as well as the cytokines, T cell subsets and humoral mechanisms involved in enterocyte apoptosis has provided numerous possibilities for dietary and pharmacological manipulation at various levels in the inflammatory cascade. The quality of life of the coeliac disease patient may be improved by enzymatic or immunological therapies which may allow intestinal mucosal remission to be achieved with less stringent dietary gluten restriction making the coeliac diet more varied and palatable.

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