

University of Nevada, Reno

**Celiac Disease: Gliadin Peptides Activate Immune System through  
CXCR3-dependent binding, FPR1 binding, and p38 MAPK Pathway  
Upregulation**

A thesis submitted in partial fulfillment  
of the requirements for the degree of

**BACHELOR OF SCIENCE, BIOCHEMISTRY AND MOLECULAR BIOLOGY**

by

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We recommend that the thesis  
prepared under our supervision by

**STEVEN J. CHACKO**

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### *Abstract*

Chapter 1 of this paper is an overview of celiac disease (CD) that covers the clinical features of the disease including pathophysiology, symptoms, risk factors, treatment, etc. The condition is caused by ingestion of gluten to genetically predisposed individuals. A fragment of gluten, gliadin, initiates multiple immune responses which result in the intestinal damage and a multitude of intestinal and extra-intestinal symptoms.

Chapter 2 takes an in-depth examination of a specific fragment, gliadin. Several laboratory studies have been completed which evaluate the mechanisms behind how gliadin triggers immunological responses such as through cytokine production, neutrophil migration, and T-cell maturation. Multiple experiments via rats have been used to discover the proteins, including CXCR3 and FPR1, and the pathways, p38 MAPK, which are used by gliadin fragments to induce inflammatory responses. Research that is focused on these gliadin-induced pathways can be potentially used reduce the risk and symptoms of CD development.

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## **CHAPTER 1**

### **A Broad Overview of Celiac Disease**

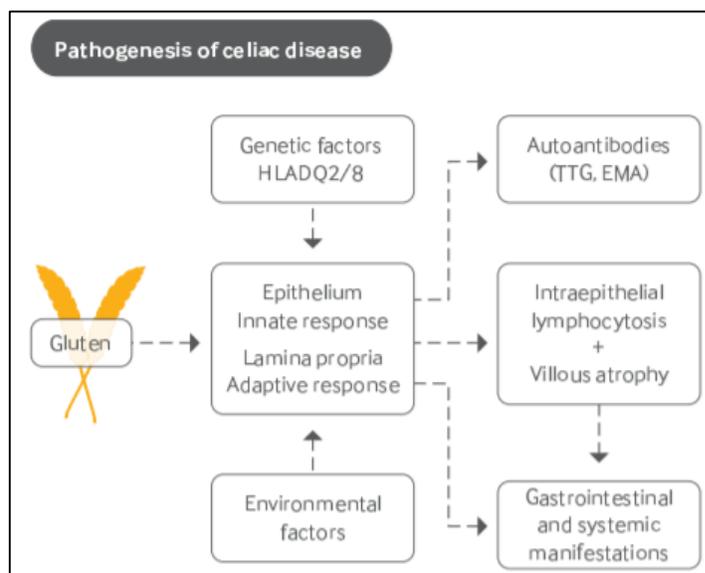
#### **Introduction**

In the past decades, multiple gluten-related disorders have started to arise, of which Celiac disease (CD), or coeliac disease, is one of the most prominent. Celiac disease is an autoimmune condition during which normal dietary gluten can play a pathogenic role to cause issues with the small intestine. Diagnosis of the disease can involve a combination of lab work, genetic testing, and intestinal biopsies. Gluten has been an integral part of the traditional diet as it is contained in wheat, rye and barley. Gluten is composed of two main fragments, gliadins and glutenins. Gliadins are soluble and are classified based on their primary structure into alpha, beta, gamma, and omega types. Glutenins are poorly soluble and are classified as either high or low molecular weight. Specifically, the fragments from gliadin trigger a T-cell mediated autoimmune reaction in the small intestine which results in an inflammatory cascade, intestinal damage, and malabsorption.

## Clinical Features

### Pathophysiology/Pathogenesis

Celiac disease is the combination of a triad of factors as shown in Figure 1. It requires a genetic predisposition, environmental factors which make the patient susceptible to having the disease, and gluten to cause symptoms of the disease. Gluten proteins are rich in both glutamines and prolamines which make them difficult to fully digest by gastrointestinal

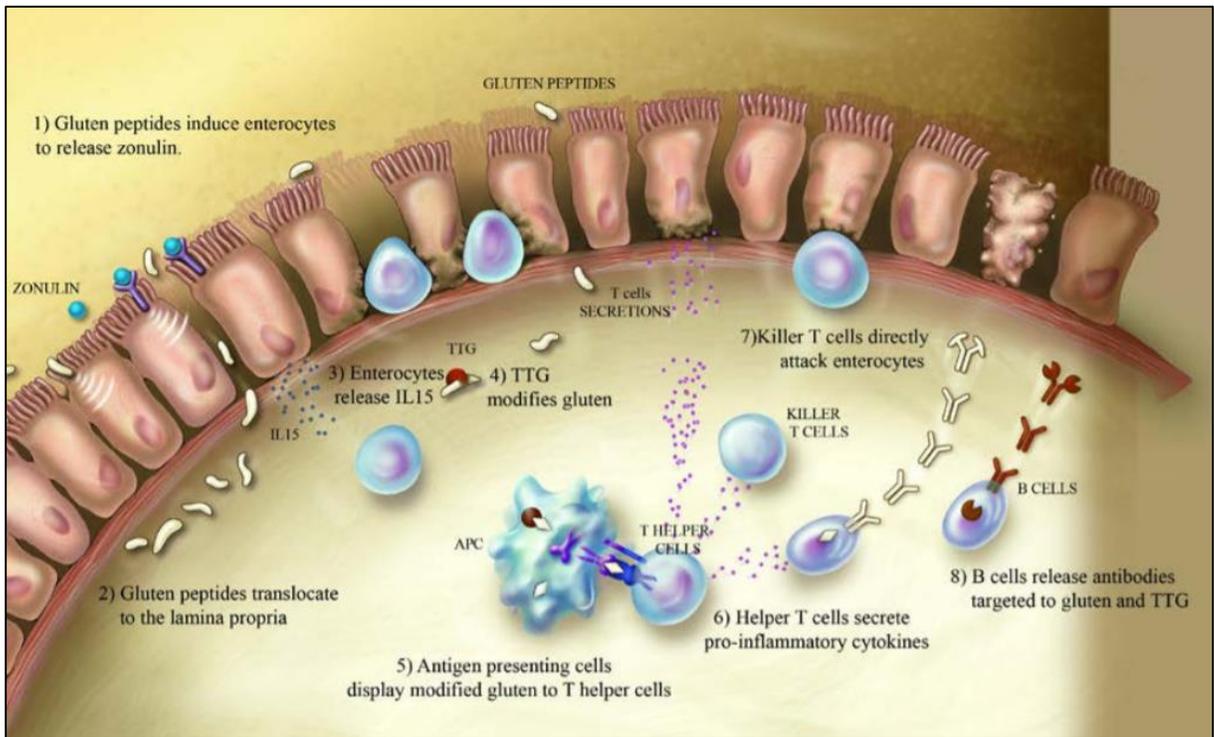


**Figure 3: Pathogenesis of Celiac Disease:** Celiac Disease is caused by a triad of factors resulting in innate and adaptive immune responses. *Source: Lebwohl et al.*

peptidases, resulting in peptides as large as 33 amino acids long.<sup>2</sup> Fragments from the gliadin component of gluten play the most important part of CD. Specific gliadin peptides can react with CXCR3 receptors on the apical side of the epithelium which causes zonulin release to dissociate the intestinal tight junctions.<sup>3</sup> These fragments are then able to pass through the epithelial border into the connective tissue underneath, the lamina propria. From there, the adaptive and the innate immune response are both triggered.<sup>2</sup>

The partially digested gliadin peptides cause the release of IL-8 and IL-15 from epithelial and lamina propria dendritic cells by interacting with the small intestine mucosa. IL-8 results in the immediate involvement of neutrophils within the lamina propria. IL-15 induces apoptosis within the intestinal lining using Natural Killer Group 2D+ (NKGD+) cells<sup>3</sup> by upregulating the NK receptors on the cytotoxic epithelial cells.<sup>2</sup>

Gliadin in the lamina propria is deamidated by tissue transglutaminase (TTG)<sup>3</sup> which increases its immunogenicity. They bind to the HLA DQ2/DQ8 molecules on antigen presenting cells and then react with CD4+ T-cells which recognize the gliadin peptides.<sup>2</sup> The peptides can interact with macrophages and dendritic cells in the submucosa to drive the adaptive Th1/Th17 immune response. The response causes the cascade of pro-



**Figure 4: Simplified Mechanism of Gliadin:** Gliadin fragments cause a cascade of events which result in enteropathy. *Source: Serena et al.*

inflammatory cytokines IFN- $\gamma$ , TNF- $\alpha$ , and IL-17. These increase intestinal permeability by weakening the tight junctions further and cause intestinal mucosa damage.<sup>3</sup> The inflammatory cascade releases tissue damaging mediators which cause injury to the epithelium.<sup>2</sup> The pathway is simplified as shown in Figure 2.

## Symptoms

The symptoms can vary between children and adults and, thus, age must be taken into consideration. In children, the common presentations are due to recurrent abdominal pain, growth issues, and are part of high risk groups such as family members with an autoimmune disease. Diarrhea has only been found in about 10% of cases, mainly in the extremely young pediatric patients. Adults are similar in that they can have diarrhea, abdominal pain,<sup>2</sup> weight loss, or failure to thrive,<sup>3</sup> but it is not a major indicator of CD. Instead, they develop many extra-intestinal symptoms such as anemia, osteoporosis, dermatitis herpetiformis, neurological or psychiatric problems, infertility, aphthous stomatitis, and other vitamin deficiencies,<sup>2</sup> cerebellar ataxias, peripheral neuropathy,<sup>1</sup> chronic fatigue.<sup>3</sup> These deficiencies in certain nutrients (iron, vitamin B12, calcium, etc.) can be due to selective malabsorption of micronutrients.<sup>1</sup>

## Epidemiology

The prevalence of CD has been found to be around 1% of the general population, with serology-based studies reporting a higher prevalence than those which also require a small intestinal biopsy.<sup>2</sup> A considerable number of those patients are predicted to be both undiagnosed and untreated.<sup>1</sup> The disease has been found to be more prevalent in Caucasians than either African-Americans or Hispanics within the US with a prevalence of around 0.7-0.8%. On the other hand, Europe varies with the UK and Germany having a low prevalence and Sweden and Finland having an estimated rate around 2-3%. China and sub-Saharan Africa have been found to have a very low prevalence of CD.

Multiple factors have been evaluated in the prevalence of the disease. Age is a factor with children and adults displaying a similar prevalence, despite expecting an increasing trend with age. Women are more commonly diagnosed with CD than men, and the disease is very uncommon in young men. Most patients diagnosed with CD are at a normal BMI with 15% being underweight and the same amount being overweight.<sup>2</sup> First-degree relatives of CD-diagnosed patients have a risk of 10-15% of developing CD in their lifetime.<sup>3</sup> Second-degree relatives also have an increased risk but not as substantial as first-degree relatives. Several studies have examined the increasing prevalence of celiac disease over the past few decades.<sup>2</sup> North America and the Europe were both evaluated to have the sharpest increases in CD.<sup>3</sup>

### Genetics

The human leukocyte antigen system (HLA) is the most well-known contribution to the CD. The major histocompatibility complex (MHC) class II HLA DQ2 and DQ8 have been found to make the patient most susceptible to the development of CD.<sup>3</sup> 95% of CD patients have the HLA-DQ2 heterodimer while the other 5% are carriers for HLA-DQ8. Thus, either of the two HLA risk alleles are necessary to develop CD but there are environmental factors which must trigger the actual onset.<sup>1</sup> There have also been genetic links not tied to the HLA genes such as those involving CTLA-4 and myosin 1XB, IL2, and IL21. Concordance between monozygotic twins has only been about 85% which means there are environmental factors which also influence CD development.<sup>2</sup>

### Environmental Factors

As expected, gluten is the most obvious environmental factor that causes CD. They have found that areas where gluten is an integral part of the diet, such as areas in Africa and Sweden in the 1990s, that more than 1% of the general population has CD. In terms of infant feeding patterns, there has been no research that has definitively found an association between breastfeeding patterns, age of gluten introduction, or CD onset. It has also been found that babies which undergo an elective cesarean section (C-section) have a slightly higher risk of developing CD which could be due to lack of exposure to bacterial gut flora during pregnancy. A normal pregnancy or an emergent C-section had no association with CD development.<sup>2</sup>

Other possible factors include viral infections which are commonly linked to immune-mediated diseases, but the evidence has been largely inconclusive. Multiple studies examined a link between exposure to GI infection in early life and later development of CD, but they had multiple issues which make a solid conclusion difficult. Smoking has had a protective link against CD in the UK, but majority of studies have found minimal association, so the current data is inconclusive. Some drugs and antibiotics have been linked to CD development but there are possible issues due to these medications potentially being used to treat the symptoms, rather than causing the onset of the disease. However, one study has examined the possible protection of *H. pylori* when used to colonize the gut.<sup>2</sup>

## Diagnoses

Diagnosis of CD is based on a combination of the patient's history, serological testing, and duodenal biopsies. Even when asymptomatic, testing is advised if in a high-risk group such as 1<sup>st</sup> degree relatives of CD patients, patients with an autoimmune disorder as such as type 1 diabetes, and patients with Down's syndrome. Serological testing can vary for age due to the sensitivity of detection. Above 2 years of age, the anti-transglutaminase IgA antibody (TTG) is recommended due to its low cost and high reproducibility. The test can function with around a 95% specificity. Another option for serum testing is the anti-endomysium antibody (EMA) which is more specific around 99% and can be used as a confirmatory test. The main reason this is not the preferred method is because it requires expensive substrates and requires an operator to interpret the pattern. Below 2 years of age, a deamidated gliadin peptide (DGP) IgA and IgG are used in combination with the TTG IgA. If the serum results are positive, duodenal biopsies are strongly recommended for the diagnostic evaluation of CD. Multiple biopsies are recommended to prevent a false negative as CD damage in duodenal mucosa is not distributed equally across the epithelium. If there is evidence of CD, the expected results include an increase in intraepithelial lymphocytes and villous atrophy. However, if there is just lymphocytic infiltration without any villous atrophy, the finding is non-specific and requires more results.<sup>1</sup>

This pathway is not always appropriate depending on the patient's particular scenario. In some cases, it is recommended to rule out an IgA deficiency which occurs in up to 2% of CD patients as it would result in a false negative. In these cases, a TTG IgG, rather than IgA, should be tested. In cases where serology tests are negative, but the

biopsy indicates villous atrophy, further evaluation is needed. Some patients also present with a gluten-free diet (GFD) already in place and thus would likely not show any histological or serum changes. In these cases, the patient should have genetic testing for the presence of HLA DQ2/DQ8 due the strong association with CD. If those results are positive, the patient will be gluten challenged under medical supervision to determine if there are new serological and histological changes. In terms of how to gluten challenge, the dosage and length is not fully clear with the current plans ranging from 2 to 8 weeks and doses of 3g to 10g. If the genotyping results are negative, CD can be excluded as a possibility.<sup>1</sup>

### Treatment

Currently, the main treatment option is a lifelong gluten-free diet (GFD) to prevent significant effects of CD. After a conversion to such a diet, symptoms begin to resolve within around 4 weeks. However, a full serological normalization takes multiple months to a year and a full histological normalization or improvement takes even longer. The median time of one study was 3.8 years before the intestinal villi reached a normal villous height. Complete intestinal recovery is not a universal trend, but this may be due to certain groups having worse adherence to a completely GFD.<sup>2</sup> Strict compliance in GFD can fail to induce complete improvement in around 7%-30% of all patients.<sup>3</sup> It was found that patients with a persistent villous atrophy did not have a difference in overall mortality, cardiovascular outcomes, or obstetric complications but had a higher risk of fractures and lymphoproliferative disorders. Even if feeling asymptomatic, a follow-up

intestinal biopsy is more sensitive than serology-based studies and could be used as a risk tool to determine if the GFD is working appropriately.<sup>2</sup>

This diet has its limitations as well due to the difficulty maintaining a completely free diet. Finding gluten-free options of various foods tends to be more expensive and some options are not even available. It is difficult avoid accidental gluten exposure in many foods, sauces, etc.<sup>2</sup> Contamination tends to be an issue for compliance as the maximum amount has been recently been defined as 20 ppm.<sup>3</sup> Gluten is an integral aspect of many foods and can be very socially isolating due to the its prevalence in many different types of social settings.<sup>2</sup> Although the diet has no side effects, patients also tend to eat less of certain nutrients including fibers, iron, calcium, and folates.<sup>3</sup> Patients who have followed a GFD rate their treatment burden similarly compared to patients with end stage renal disease who also have a strict treatment regimen.<sup>2</sup>

As a result, research has been conducted into options that are non-dietary related. Current drug therapy involves the detoxification of gluten through glutenases, the enhancement of tight junctions in the epithelium, a vaccine for gluten tolerance, immune blockers,<sup>2</sup> modified gluten, zonulin inhibitors, or supplementary probiotics.<sup>3</sup> Two current drugs are evaluated in Phase II studies to act as an alternative for a GFD. Larazotidate acetate regulates tight junctions which can make the epithelial barrier in the small intestine stronger to prevent gliadin fragments from reaching the lamina propria. Another medication, ALV003, is an endopeptidase and endoprotease which reduced the amount of villous atrophy expected in CD.<sup>2</sup>

### **Body of Review**

Celiac disease affects millions of people across the globe with a multitude of symptomatic side effects. Gliadin is the main component of gluten which induces a variety of symptomatic side effects in CD patients. Diagnosis can involve a combination of factors that range from serum testing, a biopsy for confirmation, and genetic testing in ambiguous cases. While there is no current cure, treatment of a GFD can slightly return the intestinal histology to normal and reduce most symptoms. Current research is largely focused on the immune pathways which gliadin activates and its ability to penetrate the intestine.

### **Conclusions**

Celiac disease is becoming increasingly understood as research has elucidated certain pathways that gliadin is involved in. Though not a fatal disease, treatment is very rigorous and currently requires a lifestyle commitment to prevent symptoms. Thus, a better understanding of the molecular pathways allows for inquiry into medicine that can reverse the symptomatic effects of the disease.

## **CHAPTER 2**

### **A Molecular Analysis of Celiac Disease: Gliadin peptides activate immune system through CXCR3-dependent binding, FPR1 binding, and p38 MAPK Pathway upregulation**

#### **Introduction**

Gliadin is a fragment of gluten that is unable to be fully digested within the small intestine. The fragment can bind to a CXCR3, a chemokine receptor, which subsequently causes zonulin-mediated tight junction complex (TJ) disassembly in the epithelial wall. This disassembly of the junction causes a transient increase in intestinal permeability regardless of if the patient has the disease. However, the duration of the increased permeability varies between healthy and CD patients.<sup>4</sup> The CXCR3 receptor is expressed higher in CD patients than non-CD individuals in the intestinal lumen which could explain increased symptoms. The breaking of the tight junction in the intestinal wall<sup>5</sup>, which is responsible for monitoring paracellular solute movement,<sup>4</sup> allows the large peptides fragments of gliadin to pass through and enter the lamina propria. Once inside the submucosa, gliadin triggers a cytokine response in both healthy and CD immune cells.<sup>5</sup> Interleukin (IL)-8 is a well-known cytokine that is released, which attracts neutrophils and is a chemoattractant.<sup>4</sup>

Genetically susceptible people for CD have the human leukocyte antigen HLA-DQ2 or -DQ8 molecules. When gliadin fragments cross into the submucosa, they encounter DQ2- or DQ8-restricted CD4+ T cells. After being deamidated, the fragments have an increased binding to the HLA molecules which are on antigen-presenting cells (APCs), including dendritic cells (DC) and macrophages. APCs are immature in

peripheral tissues and continually sample antigens until they encounter a signal molecule to become a functional APC that stimulates T cells. They migrate to lymph nodes and form complexes of major histocompatibility complex (MHC) molecules. The ability of the DC to activate a naïve T cell is completely dependent on its ability to migrate towards a lymph node.<sup>6</sup>

Migration is directed by a chemokine receptor, CCR7, which is regulated by two other chemokines, CCL19 and CCL21. Those two ligands are responsible for guiding the DCs properly. The CCR7 receptor can also be activated by other signals including prostaglandin E2 (PGE<sub>2</sub>) whose mechanisms are not fully known. However, not everything that activates DCs will induce full migration as alterations of the cytoskeleton and expressional changes are needed for the cell to pass. Immature DCs have shown to be at low-speed and interact with a dense extracellular matrix component, podosomes, which prevent them from easily migrating to the lymph nodes. Podosomes are actin-dense structures which prevent mobility of the cells. Mature cells will weaken the cellular matrix and have dendrites which allow them to scan for naïve T cells.<sup>6</sup>

The main limitation of the current research is that it is mainly aware of the multiple immune responses that are induced by gliadin in the epithelium. However, new research is evaluating the downstream effect of gliadin and the specific mechanism that causes things such as cytokine release, neutrophil localization, and T-cell production. The goal of this paper is to analyze the various mechanisms by which gliadin can induce an immune response through specific receptors or pathways.

## **Induction of Interleukin-8 (IL) release through chemokine receptor CXCR3-dependent binding**

### Purpose

The goal of this study was to evaluate the role of the CXCR3 receptor in the inflammatory response to gliadin using cytokine analysis.

### Materials and Methods

#### *Blood samples*

Peripheral blood mononuclear cells (PBMCs) were extracted from the blood of healthy controls (HC) and CD patients in remission on a gluten-free diet (CD-GFD).

#### *PBMC Analysis*

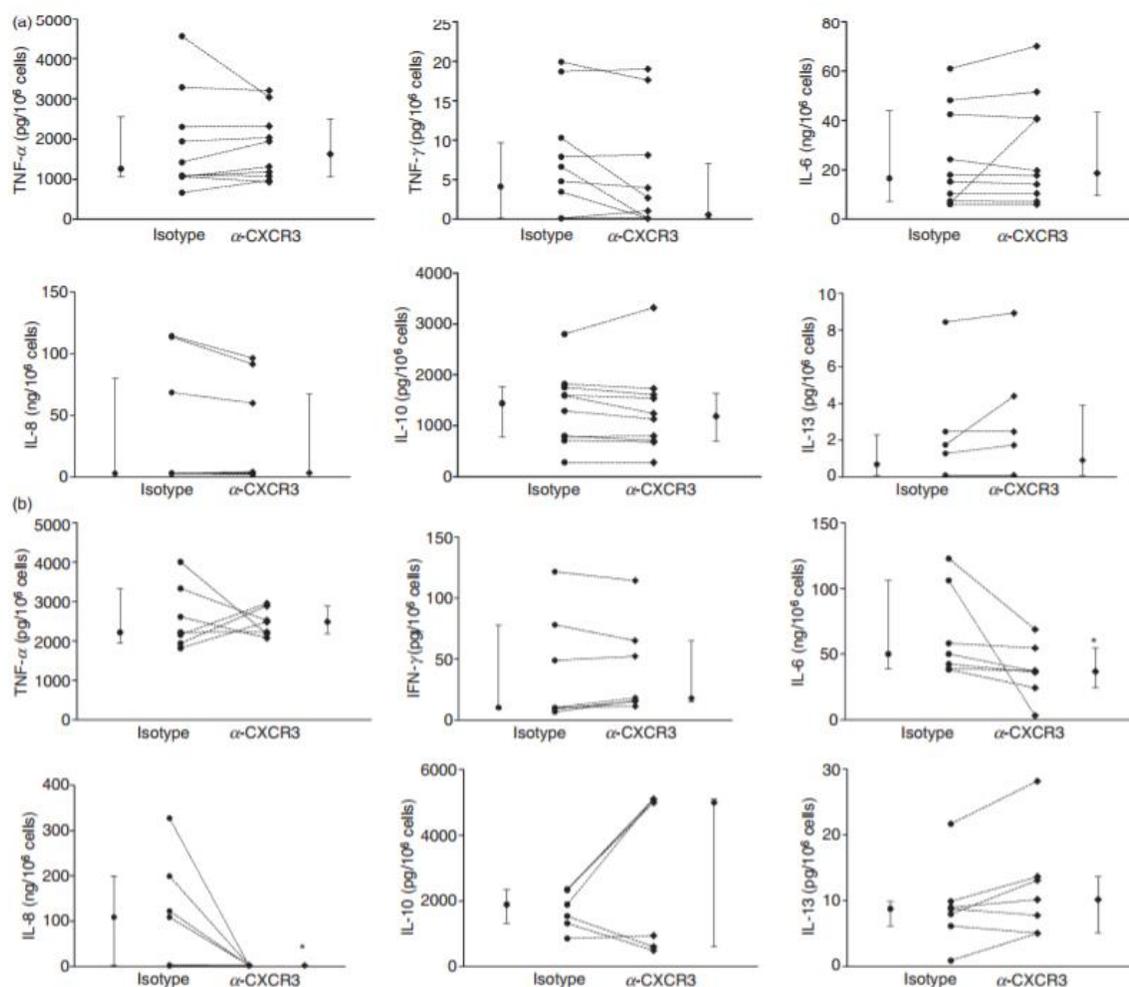
Gliadin was digested using pepsin and trypsin (PT-gliadin) to replicate normal food digestion within the GI tract. To measure concentrations of chemokines, the supernatants were analyzed with a chemokine antigen-linked assay. A blocking anti-CXCR3 antibody was used to compare the effect of the receptor on the two different conditions.<sup>5</sup>

### Results

Samples of the PBMCs from both conditions were cultured with PT-gliadin or medium to evaluate the relative production of cytokines. Regardless of clinical condition, PT-gliadin was found to induce general cytokine production across all the cytokines. As shown in figure 3, CXCR3 played a unique role in CD samples in that IL-8 and, to a lesser extent, IL-6 had significantly less production after CXCR3 was blocked. Most notably in IL-8 is that production went to a complete zero in all samples suggesting that CXCR3 is integral for that specific cytokine. On the other hand, there was no difference

in cytokine production in the PBMCs from the HC regardless of CXCR3 availability.

These results suggest that PT-gliadin induced IL-8 expression in CD-GFD patients relies heavily on interactions with the CXCR3 enzyme but not in HC patients.<sup>5</sup>



**Figure 3: Pepsin-Trypsin-digested gliadin (PT-Gliadin) induced production of IL-8 in a CXCR3-dependent manner.** PBMCs were incubated with a blocking anti-CXCR3 antibody and were subsequently treated with medium or PT-gliadin. The supernatants were assayed for the above cytokines, IL6, IL-8, IL-10, TNF- $\alpha$ , Interferon- $\gamma$ , and IL13. (A) The PBMCs were obtained from healthy controls. (B) The PBMCs were obtained from patients with celiac disease who were currently on a gluten-free diet. The dots are representative of a single sample. *Source: Lammers et al., 2011*

## **Induction of Neutrophil migration through Formyl Peptide Receptor 1 (FPR1) binding**

### Purpose

The goal of this study was to understand the mechanism behind the early involvement of neutrophils after exposure to PT-gliadin.

### Materials and Methods

#### *Materials*

Blood was collected from anonymous healthy donors and PT-gliadin was prepared similarly to the experiment above. PT-zein, a random unrelated protein, and drinking water were both used as reference controls. Lys-green fluorescent protein (GFP) transgenic mice were utilized.

#### *Intravital Microscopy*

The luminal side of the duodenum was surgically exposed while preserving mice blood flow and lymphatic vessel continuity. Images were acquired every 15 seconds for 3 hours. This allowed for in vivo intestinal examination for immediate neutrophil recruitment.

#### *Gavage Experiments*

Wild-type of Lys-GFP mice were administered with one of the three conditions (PT-gliadin, PT-zein, or drinking water). The mice were sacrificed, and the tissue was analyzed for ZO-1 with immunofluorescence microscopy. The tissue samples were also run through flow cytometry to reveal variations in cell composition of the sample.

#### *Interleukin Production*

Tissues from the mice were incubated with PT-gliadin and the supernatants were tested for IL-8 using ELISA at 1, 2, 4, and 24 hours.

#### *Chemotaxis Assays*

The EZ-TAXIScan chemotaxis assay was used to evaluate neutrophil migration when treated with PT-gliadin, fMET-Lue-Phe, PT-digested water, and PT-zein. Images were taken every 15 seconds for 30 minutes and the speed was tracked for each cell.<sup>4</sup>

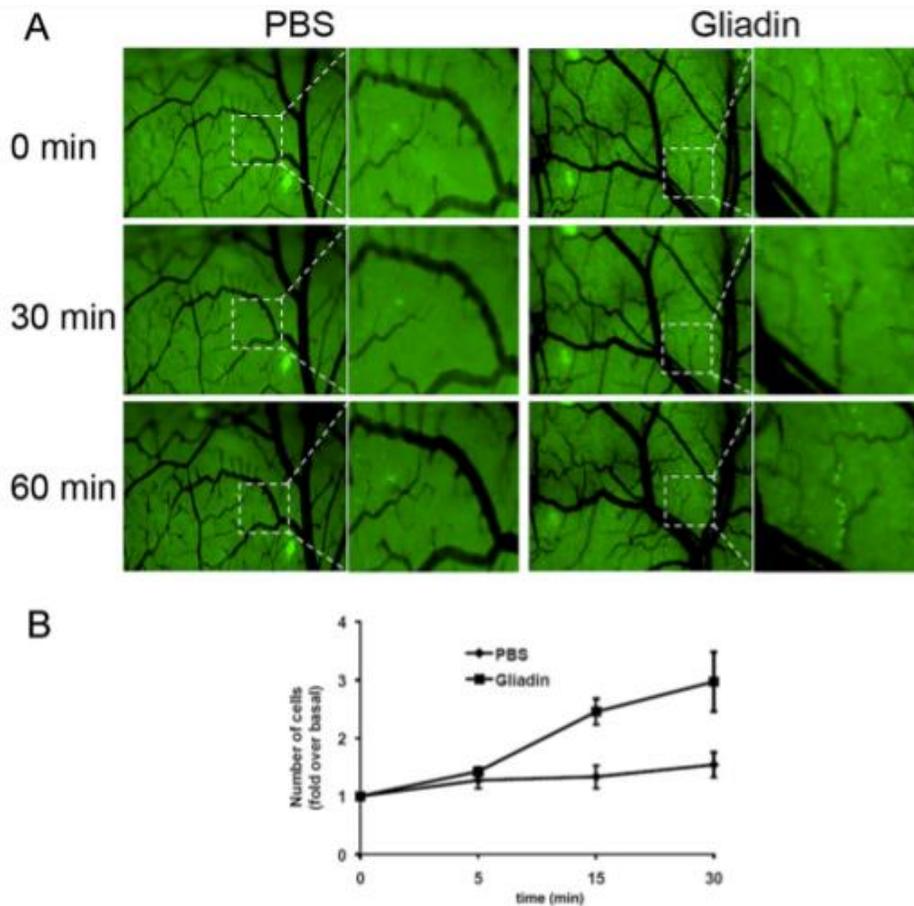
#### Results

The initial experiment confirmed the idea that PT-gliadin would induce rapid migration of neutrophils to the intestine. The results showed that within 30 minutes of injection, recruitment of neutrophils started. The control, in comparison, had no significant neutrophil migration after injection (Figure 4). Then, the lamina propria was examined to determine if the PT-gliadin was disassembling an important junctional protein, zonula occludens (ZO)-1 and recruiting neutrophils inside over time. Using flow cytometry, an increase of CD11b+Ly6G+ cell subset was found in the lamina propria which contains neutrophils and macrophages and ZO-1 dissociation was seen.

It has been previously established that gliadins induce the production of interleukin-8 (IL-8) which is a known chemoattractant for neutrophils. Thus, the next goal was to understand if the rapid migration of neutrophils occurred secondary to IL-8 production. Within a 2-hour time frame, no IL-8 production could be measured but rather took up to 24 hours. However, the previous experiment indicated that the rapid migration occurred within 30 minutes of injection. These results indicate that the sudden influx of cells is induced directly by gliadin rather than IL-8.

The next logical step is to examine if PT-gliadin has these chemoattractant properties that allow it to cause neutrophil migration. The results showed that PT-gliadin was able to induce chemotaxis on mice neutrophils with the same strength as another chemoattractant, fMet-Leu-Phe (fMLP). In a repeat study on humans, they found similar results that it was as effective as fMet-Lue-Phe.

CXCR3 was tested with the same assay to see if neutrophil recruitment would occur even in mice with the receptor protein knocked out. PT-gliadin was able to induce a similar chemotactic response in either condition showing that neutrophil recruitment is independent of CXCR3. fMLP is recognized by the formyl peptide receptor (FPR-1) and the same assay was again run to determine if that receptor played a role in the neutrophilic migration. PT-gliadin-induced chemotaxis was found to rely on the utilization of FPR1. An antagonist of FPR1 was used which completely prevented any neutrophilic migration that was previously induced by PT-gliadin.<sup>4</sup>



**Figure 4: In-vivo injection of PT-gliadin causes an immediate migration of neutrophils.** (A) Lys-GFP were imaged at 30-minute intervals in the duodenum. The bright green dots are the fluorescently tagged neutrophils. (B). The recruitment was quantitative analyzed using ImageJ to analyze the number of bright green spots. The PT-gliadin treated cells exhibited faster neutrophil recruitment in 15-minute intervals.  
*Source: Lammers et al., 2015*

### Induction of Dendritic cell migration via the p38 MAPK pathway

#### Purpose

The purpose of this study was to examine the effects of gliadin fragments on dendritic cell (DCs) migration, a prerequisite for later T cell development.

## Materials and Methods

### *Materials*

Gliadin digestion was conducted in a pepsin-agarose gel and centrifuged. PBMCs were isolated from donors and the monocytes were isolated. They were used to generate DCs and harvested for later use.

### *DC Migration*

Transwell assays were filled with medium and were treated with CCL19, CCL21, or neither in the lower chamber. The upper chamber was composed of DCs treated with gliadin fragments or immature DCs (iDCs). The cells were stained after 2 hours and analyzed for migration of cells.

### *DC Cytoskeleton*

The DC cytoskeleton was imaged using fluorescence microscopy with staining for F-actin and for the protein vinculin. The DC cells were treated with the appropriate condition.

### *RT-PCR for mRNA analysis*

CCR7 and COX-2 were both analyzed using RT-PCR after the mRNA was extracted from the cell.

### *CCR7 Surface Expression*

The surface expression was analyzed after the DC was incubated and then run through a flow cytometer. The cell viability was assessed, and the fluorescence was analyzed.

### *COX-2 Protein Expression and PGE<sub>2</sub> Production*

DCs were treated with gliadin digest and allowed variable time to incubate. The supernatants from the cell cultures were extracted and were examined with EIA for PGE<sub>2</sub> production. The cell pellets were used for COX-2 protein expression.<sup>6</sup>

## Results

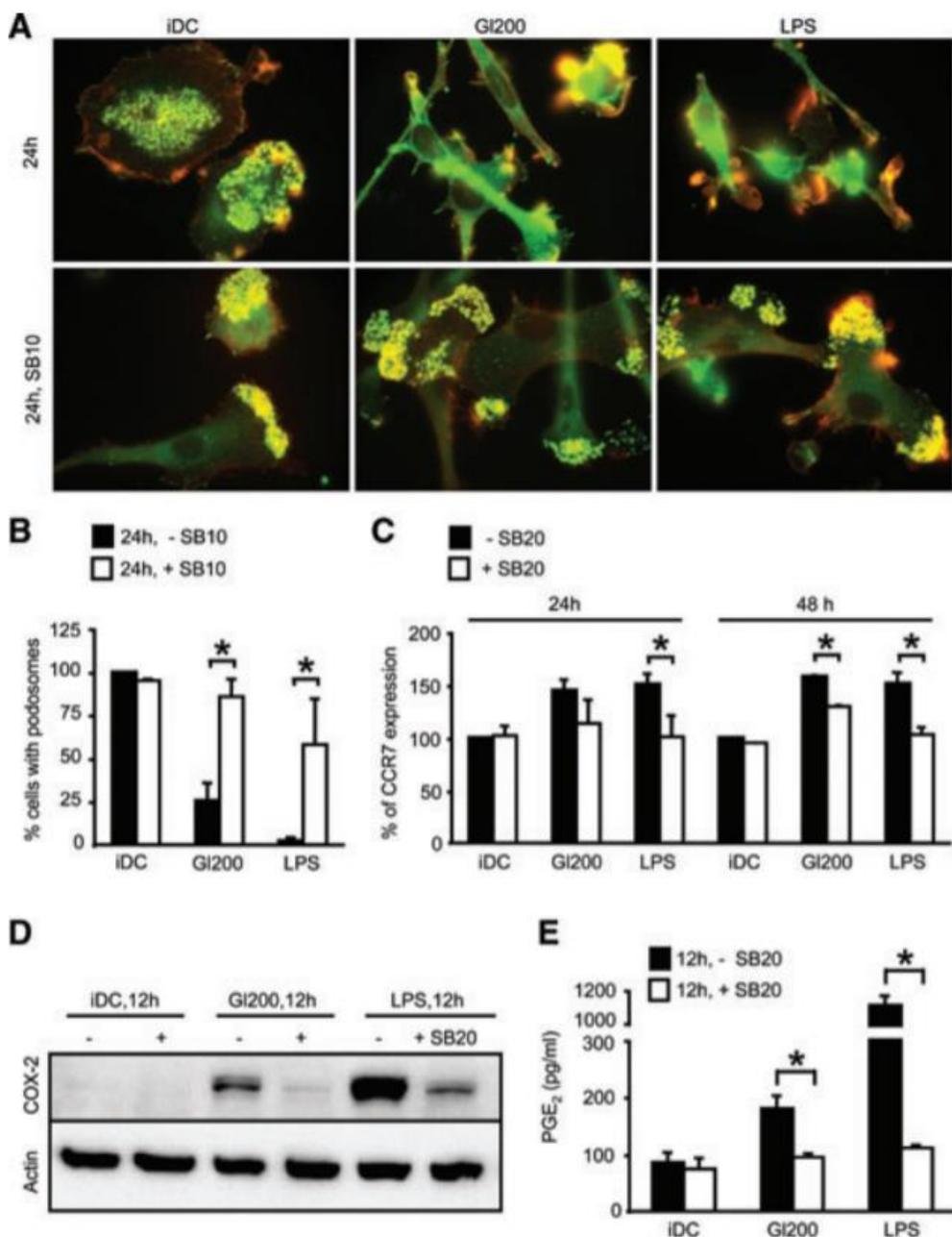
The initial experiment was to confirm whether gliadin fragments affect DC migration and if they were affected by the guiding chemokines CCL19 and CCL21. Interestingly, gliadin itself would cause spontaneous migration based on dosage and the addition of the chemokines would amplify the number of migrating DCs. The control group had minimal migration even after addition of the chemokines which indicates gliadin causes spontaneous migration and enhances chemokine response. As these ligands are linked to CCR7, gliadin fragments were found to upregulate the surface expression of the receptor after 24 hours compared to the control.

The gliadin fragments were able to assist in migration by causing cytoskeletal changes facilitating faster migration. After treating cells with gliadin fragments, the podosomes were significantly more dissolved with more dendrite protrusions compared to the control group. Thus, gliadin was able to facilitate the cytoskeletal alterations necessary for mobility.

Knowing the CCR7 receptor was overexpressed led to experimenting another activating factor, prostaglandin E2. The rate-limiting enzyme in its synthesis, COX-2, and PGE<sub>2</sub> were found to be upregulated after treatment with gliadin with a temporary increase in mRNA before returning to normal. However, PGE<sub>2</sub> synthesis, while induced by gliadin fragments, was not as significant as the upregulation from the control

treatment. Thus, gliadin mainly promotes COX-2 formation which, in turn, results in increase PGE<sub>2</sub>.

The next step was to evaluate the mechanism of gliadin fragments by examining the p38 MAPK pathway. An inhibitor of that pathway, SB203580, was used to evaluate how it would alter the results of the previous experiments. As seen in Figure 5, when the p38 MAPK pathway was inhibited, there was a loss of the various mechanisms noted above such as cytoskeletal changes, CCR7 up-regulation, and increased PGE<sub>2</sub> synthesis. Thus, it was concluded that the DC migration induced by these various control points are heavily controlled by the p38 MAPK pathway.<sup>6</sup>



**Figure 5: Gliadin fragments signaling is conducted through the p38 MAPK pathway.** Immature dendritic cells (iDC) were treated with gliadin fragments at 200  $\mu\text{g/ml}$  (GI200) and lipopolysaccharides (LPS) and were then evaluated with a p38 MAPK blocker, SB2035810 (SB10). (A, B) DCs were plated and the F-actin was stained red and the vinculin stained green. The results are shown 24 hours after treatment with the appropriate condition and the number of cells containing podosomes were measured in B. (C) Cells were treated with the appropriate condition for 24 to 48 hours and the CCR7 expression on the surface was measured using flow cytometry. (D, E) Cells were treated with the appropriate condition for 12 hours and COX-2 protein levels were analyzed

using a Western blot. The supernatant from the culture was used to evaluate the expression of PGE<sub>2</sub> in E. *Source: Chladkova et al., 2011*

## **Discussion**

The studies conducted evaluate the gliadin fragment effects on multiple factors of the immune response in celiac disease. The implications of this are that PT-gliadin potentially has a different set of pathways downstream of CXCR3 between the two variable conditions. Due to CD intestinal mucosa having higher expression of CXCR3, the PT-gliadin induced IL-8 overexpression could cause the neutrophil migration and the cascade of events leading to CD.

The second study strengthens the notion that PT-gliadin is recognized as a foreign threat and itself causes a sudden accumulation of neutrophils within the intestine. The protein has a direct chemoattractant effect which causes the neutrophils to migrate. In addition, gliadin has been shown to induce DC migration through the p38 MAPK pathway. The conclusion of these experiments indicate that gliadin promotes the generation of T cells, thereby adding another level of immune response in celiac disease. This highly complex peptide still requires more studies to be conducted in order to elucidate its full functional role in celiac disease pathogenesis.

Future research includes a deeper understanding of the CXCR3 dependency in upregulating IL-8 production, as well as the differential mechanisms between CD and healthy use of the receptor. Understanding that gliadin peptides regulate FPR1 could also be an area of analysis. The actual function of neutrophils in the immune response, whether it be productive or destructive, is also vague and requires more work.

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