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Celiac Disease Pathogenesis: The Proinflammatory Cytokine Network

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ABSTRACT

In susceptible individuals, the adaptive response, mediated by the activation of antigen-specific T lymphocytes, drives a proinflammatory response, which ends in an immune-mediated enteropathy characterized by villous atrophy, crypt hyperplasia, and recruitment of intraepithelial lymphocytes. In addition, some gluten peptides are able to induce an innate immune response in intestinal mucosa. The molecular mechanisms and the cells involved in the initial stages of the gluten–intestinal mucosa interaction are poorly understood to date. There is evidence of a direct toxic effect of gluten peptides in several biological models. However, the failure to control the inflammatory response may be one of the factors underlying gluten intolerance in these individuals. The cytokine network involved in celiac disease is characterized by abundant interferon-γ in the intestinal mucosa. In addition, the production of interleukin (IL)-15, IL-18, and IL-21 is linked to gluten intake, which can drive the inflammatory response probably sustained by IL-18, IL-21, and perhaps IL-27 through STAT1 and STAT5 pathways, whereas neither IL-12 nor IL-23 plays a significant role in pathogenic mechanisms. Herein we describe the involvement of these activation pathways in the context of the pathogenesis of celiac disease. ***JPGN 47:S27–S32, 2008.*** **Key Words:** Celiac disease—Innate immunity—Proinflammatory cytokines. © 2008 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

Celiac disease (CD) is an autoimmune-like gastrointestinal disorder triggered by the ingestion of wheat, barley, rye, and possibly oat peptides in genetically predisposed individuals. Toxic proteins have been also widely referred as gluten, which is the cohesive protein mass obtained when wheat dough is washed to remove starch and other water-soluble components (1).

The HLA-DQA∗0501, DQB∗0201, and HLA-DQA∗0301, DQB∗0302 alleles, encoding for the DQ2 and DQ8, respectively, heterodimer molecules, are the major predisposing markers of CD known to date. These alleles are present in more than 95% of patients with CD; however, they explain only approximately 40% of the genetic susceptibility. Therefore, CD is a complex genetic disorder involving multiple chromosome regions. Some of them (CELIAC2 and CELIAC4) have also been described for inflammatory bowel disease (IBD5 and IBD6, respectively), suggesting at least partially a common disease susceptibility (2). As a consequence, multiple loci, containing common genes, appear to be involved in CD susceptibility. Although genetic analysis has discovered many markers, some with unknown biological function and others related to the immune response, the individual contribution of each one is extremely low (2).

Gluten is formed by gliadins and glutenins with high proline and glutamine content. These proteins are also called prolamins. Gliadins and glutenins are the main components of the wheat storage fraction and have been extensively used for studying the mechanism of disease. Because of their high glutamine content and specific sequence patterns, prolamins are excellent substrates for deamidation by tissue transglutaminase. Both glutamine deamidated and nondeamidated peptides are presented to T lymphocytes in the context of HLA-DQ2 or -DQ8 molecules by antigen-presenting cells in the intestinal lamina propria. Some of the deamidated peptides have a high affinity for the DQ2 and DQ8 molecules and have a

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stronger stimulatory capacity (3). In susceptible individuals, the adaptive response, mediated by the antigen-specific T cell activation, drives a proinflammatory response, mainly characterized by interferon (IFN)-γ production, which ends in an immune-mediated enteropathy, where villous atrophy, crypt hyperplasia, and increased infiltration by intraepithelial lymphocytes are the typical findings. The current treatment is a lifelong strict gluten-free diet, which results in complete remission of symptoms and recovery of the normal mucosal histology (4).

The conventional gluten-driven adaptive response does not explain other common events observed in the damaged intestinal mucosa, such as altered epithelial permeability, caused by disruption of tight junctions by gliadin peptides such as p31-49 α-gliadin (5,6). These changes, among others, are now recognized as a consequence of the activation of the innate immunity. Activation of the adaptive response and some still poorly characterized innate mechanisms determine the pathogenesis of CD (7–9).

In this scenario, toxic peptides, such as the 19-mer, trigger an innate immune response (10), characterized by the production of IL-15 by epithelial cells and lamina propria dendritic cells (11). IL-15 affects the epithelial barrier, both by increasing the permeability through disruption of the tight junctions (12,13) and by inducing enterocyte apoptosis after intraepithelial lymphocyte reprogramming into natural killer (NK)-like cells (14,15). Therefore, immunoadaptive peptides, like the 33-mer, can now reach the lamina propria, where they are presented by dendritic cells to gluten-specific T cells (16,17).

As mentioned, the consequences of the gluten interaction with the intestinal mucosa in CD are well established, but the mechanisms and the cells involved are poorly understood to date. There is increasing evidence of a direct toxic effect of gliadins, the most studied component of gluten, in several biological models. For example, it has been reported that gliadin induces rearrangements of the cytoskeleton, disassembling the integrity of the tight junctions system in a zonulin-dependent manner, in epithelial cell lines like Caco-2 cells (5,18), IEC-6 cells (12,18), or the LoVo multicellular system (19), and also in non-CD biopsy specimens challenged in vitro with gliadin (18). Gliadins are also a potent stimulus for antigen-presenting cells such as monocytes, macrophages, and dendritic cells, in both humans and mice (6,20–22). Also, gliadins inhibit DNA and RNA synthesis and induce apoptosis in Caco-2 cells (23,24). All of these reports suggest a generalized innate triggering effect of gluten by a still unknown receptor in CD as well as non-CD intestinal mucosa. In this regard, we have shown the existence of an innate IL-15 response to gliadins in both patients with CD and patients without CD by using a biopsy culture model (25), although non-CD explants do not develop a subsequent proinflammatory response. The latter observation points to an increased IL-15 sensitivity or a lack of the inflammation-controlling mechanisms in CD patients.

INITIAL STEPS IN THE PATHOGENESIS OF CD: THE ROAD TO INFLAMMATION

Different pathways are activated as a consequence of the interaction of gluten peptides with the intestinal mucosa in patients with CD. Gluten peptides drive an oxidative stress characterized by nitric oxide production through inducible nitric oxide synthase induction in enterocytes (26–29), promote the production of tissue transglutaminase and the expression of danger signals (eg, MICA-MHC class I–related chain A molecules) in epithelial cells (30), and stimulate the activation and secretion of IL-15 probably by lamina propria dendritic cells. IL-15 acts on intraepithelial lymphocytes (IELs) promoting IFN-γ production and a potent cytotoxic activity particularly by $NKG2D^+$ cells. MICA is an NKG2D ligand, which targets cells for apoptosis induction by cytotoxic lymphocytes (14,15).

It is widely recognized that IFN-γ is the dominant cytokine in the damaged intestinal mucosa. Although IFN-γ is produced even during fasting periods, IL-15 seems to be produced in waves that are likely linked to gluten intake (31). However, it is still unknown how IFN-γ production is sustained. Analysis of IL-15 in intestinal biopsy specimens in active CD during the fasting period showed no increment in the majority of the analyzed samples, but we observed higher levels of IL-18 (31). However, both active forms of IL-15 and IL-18 were induced after in vitro gluten challenge of biopsy specimens from treated patients. By immunohistochemistry, on small intestine sections from untreated patients with CD, we detected IL-18–producing cells in the epithelium of the crypts (31). IL-18 induces expression of IFN-γ by itself or synergistically with IL-12 (32,33), but it presents a paradoxical effect of inducing apoptosis (34) and protecting the integrity of epithelia by an IL-11–mediated pathway (35). We found that both IL-11 and its receptor were increased in CD (36). Cytokines of the IL-12 family (IL-12, IL-23, and IL-27) are also candidates for IFN-γ long-standing stimulus. Although IL-12 is the main inducer of IFN-γ in other intestinal inflammatory processes, the lack of IL-12 in active CD is well known (16).

These cytokines, after recognition by the specific receptor, signal through different activating transcription factor (STATs) pathways. A schematic view of the common STAT signaling is shown in Fig. 1. To evaluate the activation pathways involved in IFN-γ expression, we studied molecules related to the different STATs in intestinal biopsy specimens by macroarray technology (36).

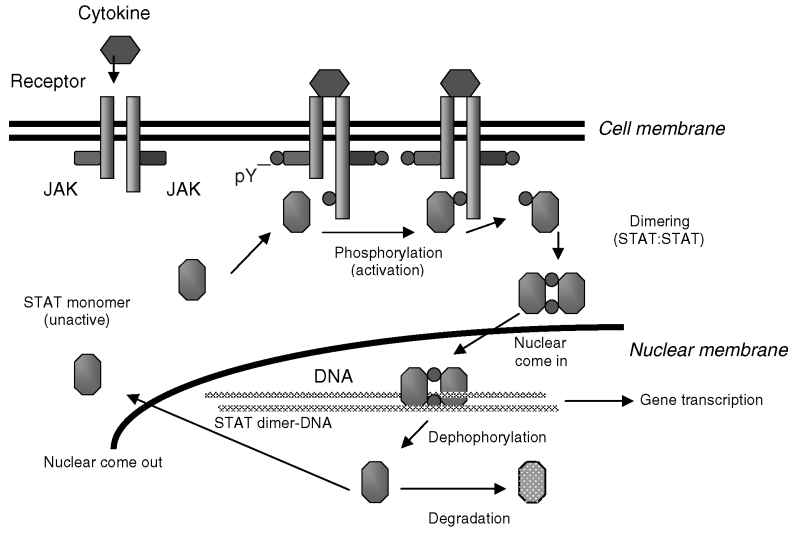

FIG. 1. Mechanism of signal transduction via STAT pathways. Cytokine binding to its specific receptor activates Janus kinases (JAK) that catalyze tyrosine residues on cytokine receptor. These are used as docks for STAT molecules phosphorylation. Phospo-STATs form dimers that can cross the nuclear membrane and bind to their specific DNA sequences and activate gene transcription.

Low-density arrays or macroarrays are more user-friendly variants than are microarrays, and they are designed specifically to study one system or pathway, exploring the expression of some tens or a few hundreds of genes. After selection of candidate genes, its expression was individually assessed by quantitative polymerase chain reaction (qPCR). All patients attended the Adult Gastroenterology Clinic at Hospital Clinico Universitario, Valladolid. Total RNA was purified using the Trizol reagent (Invitrogen, Life Tech, USA) and IL-6, IL-15, IL-18, IL-21, and IL-12 family (IL-12p35, IL-12p40, IL-23p19, and IL-27p28) and STATs (1, 2, 3, 4, 5a, and 5b) mRNAs expression were tested by q-PCR using the LightCyler instrument (Roche Applied Science, Germany) and specific primers. PCR conditions specified below. IL-15 and IL-18 analyzed as described previously (31). β-Actin mRNA levels were used as housekeeping. The results were treated with nonparametric statistical tests: medians and Mann-Whitney were used for comparison between groups and Wilcoxon test for 2 related samples statistics. In both cases, statistical significance was considered in differences with $P < 0.05$. Cytokines mRNA levels are expressed in arbitrary units. Table 1 describes the primers used in the analysis. Although we found differential expressions of STAT3 and STAT5 pathways in active CD by macroarray analysis, we could not validate these findings by real-time PCR. STAT1, STAT3, and STAT5 showed increased levels in active CD, with no statistical significance (Fig. 2). As a consequence of the wide dispersion of results, we did not detect statistical differences in all of the parameters tested. The dispersion may be a consequence of differences in

TABLE 1. *Samples and methods in duodenal and jejunal mucosal biopsy specimens of patients with active CD (altered mucosa, 27; nonaltered mucosa, 25), CD in gluten-free diet (GFD), and non-CD control individuals*

Gene	Primer forward	Primer reverse	Temperature, °C	Base pairs
Il-6	Commercial primers and Taqman probes	Applied Biosystems, USA		
Il-12p40	Commercial primers and Taqman probes	Applied Biosystems, USA		
p35	F:5′-TGTCACCGAGAAGCTGATGT -3′	F:5′-GAGGTTTCTGGCCAAACTGA-3′	68	278
Il-21	Commercial primers and Taqman probes	Applied Biosystems, USA		
Il-23p19	F:5′-AGCAGCTCAAGGATGGCACTCAG-3′	R:5′-CCCCAAATTTCCCTTCCCATCTA-3′	55	251
Il-27p28	F:5′-GCGGAATCTCACCTGCCA-3′	R:5′-GGAAACATCAGGGAGCTGCTC-3′	64	69
STAT1	F:5′-CCATCCTTTGGTACAACATGC-3′	R:5′-TGCACATGGTGGAGTCAGG-3′	56	71
STAT2	F:5′-AGGCCGATTAACTACCC-3′	R:5′-AGTGGCAGGCTTGTTC-3′	60	372
STAT3	F:5′-GCCAGAGAGCCAGGAGCA-3′	R:5′-ACACAGATAAACTTGGTCTTCAGGTATG-3′	62	75
STAT4	F:5′-ACATCCTGCGAGACTAC-3′	R:5′-ACACCGCATACACACT-3′	57	264
STAT5a	F:5′-AAGAGGTAGAAAAGATTGGG-3′	R:5′-CTGCGAGTCCTACAGC-3′	57	304
STAT5b	F:5′-CATTCGGTCCCTAGCC-3′	R:5′-CGCGCTACGTCCATTG-3′	62	345
STAT6	F:5′-GCAGTTCAACAAGGAGATCCTGCT G-3′	R:5′-TTTCCACGGTCATCTTGATGGTAGC-3′	62	455
β-Actin	F:5′- ATGGGTCAGAAGGATTCCTATGTG -3′	R:5′- CTTCATGAGGTAGTCAGTCAGGTC−3′	60	359

TABLE 2. *STAT pathways used by receptors of the different types of cytokines for signal transduction*

Ligands	STATS
Type II cytokines	
IFN family	
IFN-γ	STAT1, STAT2, STAT5
IFN-α/-β/-ω	STAT1, STAT2, STAT3-6
IL-10	STAT3
IL-19, IL-20, IL-24	STAT1, STAT3
IL-22	STAT1, STAT3, STAT5
Type I cytokines	
gp130 family	
IL-6, IL-11,OSM, LIF	STAT1, STAT3, STAT5
Leptin	STAT3
IL-12	STAT1, STAT3, STAT4, STAT5
IL-23	STAT1, STAT3, STAT4, STAT5
IL-27	STAT1, STAT2, STAT3, STAT5
C-γ family	
IL-2	STAT1, STAT3, STAT5
IL-4	STAT3, STAT5, STAT6
IL-7	STAT1, STAT5
IL-9	STAT3, STAT5
IL-13	STAT3, STAT5, STAT6
IL-15	STAT5, STAT6
IL-21	STAT1, STAT3, STAT5
C-β family	
IL-3	STAT1, STAT3, STAT5, STAT6
IL-5, GM-CSF	STAT5

genetic background encoding inflammatory response molecules and therefore related to the well-known heterogeneity of the disease.

It is known that the STAT1 pathway is activated by IL-18 and IL-27, STAT3 by IL-6 and IL-23 or IL-27, and STAT5 is related to γ-chain cytokines (IL-2 family, IL-9, or IL-21) (Table 2). All of them have strong proinflammatory activity. STAT4, the transcription factor activated by IL-12, remained unchanged (Fig. 2), in line with the observation that signaling through IL-12 is not involved in the pathogenesis of CD.

When cytokine expression was studied at the mRNA level, we found increased expression of IL-27 (p28) and IL-21 in active CD (Fig. 3), but no differential expression was found for IL-12 (p35 or p40), IL-23 (p19), or IL-6 (not shown).

IL-27 is a heterodimeric cytokine composed of p28 and EBI3 subunits, which are homologous to the p35 and p40 subunits of IL-12. It is also produced by activated antigen-presenting cells, and its receptor is expressed in macrophages, natural killer (NK) cells, and B and T lymphocytes (37). IL-27 is a potent inducer of IFN-γ production, but it has the paradoxical action of blocking the proinflammatory effect of IL-23 (38).

IL-21 is a lymphocytic cytokine that promotes the proliferation of T cells, generation of memory B cells, and activation of NK cells (39). In the intestinal mucosa, IL-21 secretion may be linked solely to lamina propria lymphocytes because there is no evidence that IELs may be also a source of this cytokine. In CD, IL-21 is produced by lamina propria lymphocytes by gluten stimulus, and it mediates STAT1-Tbet induction of IFN-γ (39). Genetic studies have shown that the IL-21 gene maps in a region linked to CD susceptibility (40). We detected a higher expression of IL-21 in patients with active CD than in patients with CD using a gluten-free diet ($P = 0.17$), but no differences were found in comparison with control individuals (Fig. 3). Another point to clarify is whether IL-21 can act as an inducer of IFN-γ production, synergistically with IL-18, or whether IFN-γ and IL-21 are induced in parallel and mutually promote the expression of each other in a loop (39).

Finally, another field to explore is the role of innate receptors in driving some of the events that have been previously described. For example, the expression of both IL-21 and IL-27 can be induced by glycan receptors and Toll-like receptors (39,41), and gluten has lectin activity. These facts may explain some of the direct toxic effects of gluten on intestinal mucosa in patients with CD.

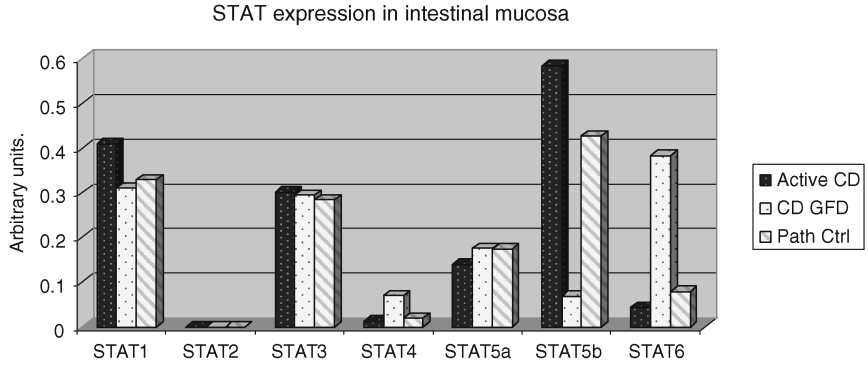

FIG. 2. Expression mRNA of STAT1-6 in small intestinal mucosa homogenates of active CD (n = 31), CD with gluten-free diet (CD GFD, n = 10), and a pathological control group (Path Ctrl, n = 15) with mild mucosal alterations ruled out CD. The values are the median mRNA expression by q-PCR in arbitrary units normalized against β-actin expression. STAT5a and STAT5b are both active isoforms, and each one is predominant in different cell lineages.

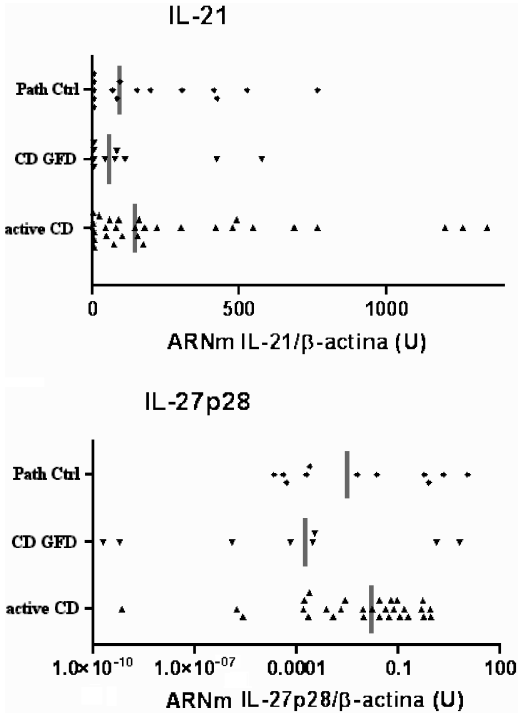

FIG. 3. Values of mRNA expression of IL-21 and IL-27 in small intestinal mucosa homogenates of active CD (n = 31), CD with gluten-free diet (CD GFD, n = 10), and a pathological control group (Path Ctrl, n = 15) with mild mucosal alterations and CD ruled out. The mRNA expression is represented by q-PCR in arbitrary units normalized against β-actin expression. Horizontal bars show the median value.

SUMMARY

In summary, gluten peptides show the property of triggering the innate immune response in intestinal mucosa. Failure to control the inflammatory response may be one of the factors underlying gluten intolerance in genetically predisposed individuals. The cytokine pattern is predominantly proinflammatory, with IFN-γ as the main effector molecule. IL-15, IL-18, and IL-21 are produced linked to gluten intake, but the inflammatory response seems to be sustained by IL-18, IL-21, and perhaps IL-27 through STAT1 and STAT5 pathways, whereas neither IL-12 nor IL-23 plays a significant role in the pathogenesis of CD.

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