

Acid-Hydrolyzed Gliadins Worsen Food Allergies through Early Sensitization

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Scope: Food allergies result from a complex immune response involving both innate and adaptive immune cells. Major proteins of wheat flour, gliadins, appear to be important allergens, and their characteristics can influence the allergic response. This study investigates the immune reaction when developing a food allergy to gliadins in native, deamidated, or hydrolyzed forms.

Methods: The immune response after one or two intraperitoneal sensitizations and after oral challenge with each gliadin form is analyzed.

Results: Results demonstrate that deamidated gliadins induce a stronger allergic reaction compared to native gliadins. Moreover, deamidation induces an earlier increase in intestinal permeability associated with more pronounced Th2 and Th17 polarizations together with an influx of antigen-presenting cells, especially cDC2.

Conclusion: Altogether, Results indicate that industrial processes such as deamidation or hydrolysis influences food allergenicity through immune modulation and helps us to develop tools to determine how these processes can influence this reaction and encourage or decrease allergic reactions.

to 5% in adults^[1,2] and is constantly on the rise, making food allergies a topical issue. Food allergies have reached worldwide epidemic proportions, and the World Health Organization (WHO) has classified allergies as the fourth global disease; thus, it is important to increase our understanding of their development.^[3] Wheat is a major component of the human diet, particularly in Western countries. Among food allergies, wheat allergy is predominant with an estimated prevalence of 0.4% based on oral challenge experiments in American and European populations.^[4] Proteins derived from wheat grain have been implicated in food allergies. In both children and adults, wheat allergy induces a variety of symptoms: atopic eczema, atopic dermatitis, chronic urticaria, anaphylactic shock, or exercise-induced anaphylaxis.^[5,6]

1. Introduction

A food allergy, or hypersensitivity, is an abnormal response to an allergen that is triggered by the immune system. The prevalence of food allergies varies from 4% to 8% in children and from 1%

Wheat is composed of two fractions: the insoluble fraction and the soluble fraction. The insoluble fraction is called gluten, and its proteins can be divided into gliadins and glutenins. Wheat, particularly the gluten, contains a large variety of proteins that can be allergens. Gluten has the particularity of forming a thick gel with great visco-elastic properties. Gluten is largely used in the food industry, mostly because of its structure. Indeed, it improves the texture and increases the protein quantity in food products. However, its density restricts its utilization as a food ingredient. To counteract this problem, various processes have been used to improve its solubility. The hydrolysis of wheat proteins is often used in the food industry to improve the emulsifying properties of the products. Thus, hydrolyzed gliadins are integrated into baked or cream-based products and in cosmetic products such as shampoo or conditioners.^[7–9] However, these processes can change the structure of the proteins, and it has been shown that some processes produce new allergens.

Murine models constitute good tools to improve our understanding of allergic sensitization mechanisms and symptom elicitation. The allergic response to wheat proteins observed after a food allergy protocol is mostly orchestrated by Th2. The sensitization to some acid-hydrolyzed wheat proteins increases the production of IgE and IgG1 and the secretion of IL-4 and IL-5 by splenocytes.^[10] In the same way, sensitization to deamidated

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gliadins induces the secretion of IgE, IgG1, and IL-4.^[11] The oral intake of gliadins or glutenins before a moderate exercise of 30 min in mice induces the production of IgE and alteration of the intestinal barrier, showing the impact of wheat protein on food-dependent exercise-induced anaphylaxis (FDEIA).^[12] For gliadins to interact with cells in the immune system, they have to overcome the intestinal barrier. Gliadin peptides cross the epithelial layer by transcytosis or paracellular transport. Paracellular transport occurs when intestinal permeability is increased, a feature that is characteristic of allergies.^[13] Gliadins have been demonstrated to increase permeability in human Caco-2 intestinal epithelial cells by reorganizing actin filaments and altering the expression of junctional complex proteins.^[14] However, how gliadins impact gut permeability linked to the allergic response remains unexplored.

Dendritic cells (DCs) are a key player in the establishment of oral tolerance and in the induction of specific immune responses. In particular, specific populations of DCs in the lamina propria are responsible for sampling soluble antigens in the intestinal lumen by extending their dendrites through the tight junctions of the intestinal epithelium and transporting them to mesenteric lymph nodes (MLNs). This process is necessary for the establishment of a specific immune response resulting in the activation of T and B cells but also in the generation of regulatory T cells.^[15,16]

Even if the allergic response is well-documented, the exact mechanisms of the immune response toward different forms of the same protein are still unclear. Indeed, how the industrial processes modifying the wheat protein impact the immune mechanisms is unknown. Our work aimed to study the effect of three forms of gliadins on the sensitization phase and the elicitation phase in a murine model of food allergy. The immune response toward native gliadins, deamidated gliadins, and enzymatic-hydrolyzed gliadins was studied at three different time points: after the first sensitization, after the second sensitization, and after the oral challenge by gavage. To investigate if different industrial processes alter the development of an allergic response, we studied the intestinal physiology, the Ig response, and the cell infiltration at the three different time points. These results will provide new data on the evolution of the immune mechanisms during the sensitization and elicitation phases.

2. Experimental Section

2.1. Animal Model

Balb/c mice from Janvier, France were used and housed in a ventilated cage system. The protocol was approved by the Ethics Committee on Animal Experimentation of Pays de Loire (CEEA) under the authorization number 4049. Wild-type, 4-week-old balb/c mice were sensitized to wheat native gliadins (NG), deamidated gliadins (DG), or hydrolyzed gliadins (HG) by two intraperitoneal injections of 10 μ g in 100 μ L of phosphate-buffered saline (PBS) plus 100 μ L of aluminum hydroxide and separated for 10 days. After one week, mice were orally administered 20 mg of NG to induce the allergic reaction. Control mice were sensitized intraperitoneally with aluminum hydroxide only and challenged with PBS.

2.2. Gliadin Preparation

Crude gliadins were extracted from wheat flour (Hardi cultivar) using the sequential procedure. Briefly, the albumin–globulin fraction of the flour was removed by several washings with saline buffer, and the flour was suspended in 70% ethanol for 1 h at room temperature (RT) to solubilize the gliadins. After centrifugation ($20\,000 \times g$ for 20 min at 41 °C), the supernatant, that is, the whole NG extract, was collected and freeze-dried. Deamidation was carried out by dispersing 20 mg of NG in 1 mL of 0.1 N hydrochloric acid. The temperature was increased to 90 °C and maintained at this level for 1 h. The reaction was stopped by neutralizing with 0.1 N NaOH. The resulting mixture was dialyzed for 5 days against water (changed every day) and centrifuged to recover the supernatant. This soluble material was then freeze-dried (DG). NG were hydrolyzed by Alcalase (ratio E/S = 1/9) over 2 h at 55 °C (HG). The reaction was stopped by heating the solution at 85 °C over 10 min and then cooling on ice.

2.3. Measure of Ear Thickness

Just before the food allergy challenge, the ear thickness of the mice was measured using a digital micrometer. One hour after gavage, the ear thickness was measured a second time. The results are expressed as the difference between the ear thickness measured after the challenge and the ear thickness measured before it.

2.4. Immunoglobulin Assay

Blood was removed by cardiac puncture 1 h after the challenge and centrifuged at 3000 rpm for 15 min. The quantification of gliadin-specific IgE, IgG1, and IgA was assayed on serum samples by indirect ELISA. Wells were coated with gliadins (NG, DG, or HG) at 5 μ g mL⁻¹ in carbonate buffer (30 mM Na₂CO₃ and 70 mM NaHCO₃) and incubated for 2 h at RT. After saturation of the wells with 0.1% PBS/Tween/0.5% gelatin for 1 h, sera were added (1:20) and incubated overnight at 37 °C. The plates were washed with 0.1% PBS/Tween, and a detection antibody directed against mouse IgE, IgG1, or IgA and conjugated to alkaline phosphatase (1:250) was added in 0.1% PBS/Tween/0.5% gelatin. Finally, an alkaline phosphatase substrate (4-methyl-umbeliferin) diluted in TRIS buffer (1:5) was added over 1 h 30 min at RT in the dark. Fluorescence intensity was measured at 440 nm with a fluorimeter as previously described.^[17,18]

2.5. Analysis of Intestinal Permeability

Pieces of jejunum were extracted from mice, washed with a Krebs solution, and mounted in Ussing chambers. The transcellular permeability was measured by horseradish peroxidase (HRP) activity over time using a spectrofluorometer.

2.6. Western Blot

For the analysis of tight junctions expression, total proteins from tissues samples stored in RA1 buffer were precipitated and prepared for polyacrylamide gel electrophoresis (PAGE) using protein precipitator and resuspension buffer (Protein solving buffer and (tris(2-carboxyethyl) phosphine) TCEP reducing agent, PSB/TCEP) from NucleoSpin RNA/Protein Kit (Macherey-Nagel, Hoerd, France), according to the manufacturer's instructions. Equal amounts of protein (50 μ g) followed by sonication with "vibracell 75 186" device (Sonics, Newton CT, USA) were separated using NuPage Novex 3–8% Tris Acetate MiniGels for Cingulin and ZO-1 and Actin or NuPage Novex 4–12% Bis-Tris MiniGels for Occludin, JAM-A, and Actin. Electrophoretic transfer to nitrocellulose membranes were realized with the iBLot 2 Dry Blotting System (ThermoFisher, Courtaboeuf, France). Membranes were processed for immunoblotting using rabbit polyclonal anti-Cingulin (1:500, Santa Cruz), mouse monoclonal anti-ZO-1 (1:200, ThermoFisher), rabbit polyclonal anti-JAM-A (1:500, Bethyl), rabbit polyclonal anti-occludin (1:500, Abcam), mouse monoclonal anti-Actin (1:10000, Sigma-Aldrich) antibodies and the relevant immunoreactive bands were quantified by actin normalization with the software Image Lab (Biorad, Marnes-la-coquette, France).

2.7. Histology

One centimeter pieces of jejunum were extracted from mice, fixed in 4% paraformaldehyde for at least 48 h, embedded in paraffin and cut and stained with hematoxylin eosin for morphological studies and inflammatory scoring. Histological images were taken with an Axio Imager M1 microscope and an Axiocam HRc (Zeiss). Sections were analyzed using ImageScope for image acquisition (Aperio Technologies, Inc.), and a cell type count for goblet cells and leukocytes was performed with 10 villi per mouse. Grading of intestinal inflammation was determined in a blinded fashion as previously described.^[19,20] Briefly, sections of mouse jejunum were individually scored based on three parameters. Inflammation was scored as follows: 0, rare inflammatory cells in the lamina propria; 1, increased numbers of cells in the lamina propria; 2, confluence of inflammatory cells extending into the submucosa; and 3, transmural extension of the inflammatory infiltrate. Crypt damage was scored as follows: 0, intact crypts; 1, loss of the basal one-third; 2, loss of the basal two-thirds; 3, entire crypt loss; 4, change of epithelial surface with erosion; and 5, confluent erosion. Ulceration was scored as follows: 0, absence of ulcer; 1, one or two foci of ulcerations; and 2, confluent or extensive ulceration. The total histological score represents the sum of all features evaluated and thus ranges from 0 to 10.

2.8. Isolation of Cells and Flow Cytometry

MLNs were collected from mice and ground. In all, 1.10^6 cells were transferred to a 96-well round-bottom plate and stimulated for 5 h with 50 ng mL⁻¹ of phorbol-12-myristate-13-acetate and 1 μ g mL⁻¹ of ionomycin (Sigma-Aldrich) together with brefeldin

A (10 μ g mL⁻¹; BD Bioscience). Cells were stained with surface markers (CD3-FITC, CD4-Alexa 405, CD25-PE-Cy7, CD11c-BV510, MHCII-APC-Cy7, SiglecH-PE, CD11b-APC, Ly6C-APC-H7, CD103-BV4, CD68-BV4, Ly6G-PE-Cy7, B220-PerCP5.5, BD Biosciences) in the presence of Fc blocker mouse CD16/CD32 antibody (BD Biosciences) and fixed in 4% paraformaldehyde (PFA). For intracellular staining, the cells were fixed and permeabilized using a Cytofix/Cytoperm kit (BD Biosciences) and stained with IL-4-PE, Foxp3-APC IFN- γ -APC, ROR γ t-PE, or IL-17 APC-H7 (BD Biosciences). Cells were analyzed on a Canto II flow cytometer (BD Biosciences). Data were acquired using Diva 8.0 software and analyzed with FlowJo X (TreeStar).

2.9. Statistical Analysis

Data were analyzed using GraphPad Prism 6.0 (La Jolla, CA, USA). Values were expressed as the mean $\pm$ SEM and compared using one-way ANOVA. In the case of a significant ANOVA, means were compared using the Mann–Whitney *U* test, and the results were corrected by Dunn's multiple-comparisons test. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Induction of an Allergic Response to Native, Deamidated, or Hydrolyzed Gliadins

To investigate whether development of allergic sensitization and elicitation to different wheat gliadin proteins is different, mice were sensitized and challenged with NG, DG, or HG using a validated protocol for food allergy (Figure 1A)^[19]. To characterize the allergic symptoms, we measured the ear thickness with a micrometer after the first sensitization, after the second sensitization, and after the oral challenge. The ear thickness measured after each one of the sensitizations was the same for all the groups, supporting the notion that it is the oral challenge that really triggers the allergic response (data not shown). Our results show a significant increase in ear thickness after the oral challenge for the mice sensitized with the deamidated form of gliadins compared to mice sensitized with NG (101 vs 80 μ m, *p* < 0.05). Moreover, no effect was observed with the hydrolyzed form compared to control mice (Figure 1B). The same results were observed when measuring the level of wheat-specific IgE in the blood of mice after the oral challenge (Figure 1C). In fact, mice sensitized and challenged with deamidated gliadins had higher levels of specific IgE compared to native gliadin allergic mice (22 vs 14 mA.U, *p* < 0.001), and hydrolyzed gliadin allergic mice showed a level of specific IgE comparable to control mice (4.5 vs 7.5 mA.U, *p* > 0.5). At the tissue level, we assessed tissue inflammation by hematoxylin-eosin staining of jejunum fractions (Figure 1D). The assessment of the histological score after the challenge showed greater inflammation for the mice sensitized with NG compared to mice sensitized with HG (4.13 vs 2.04, *p* < 0.0001) (Figure 1E). Moreover, in line with the ear thickness increase and specific IgE levels, mice allergic to deamidated gliadins exhibited stronger alterations of the intestine compared to mice

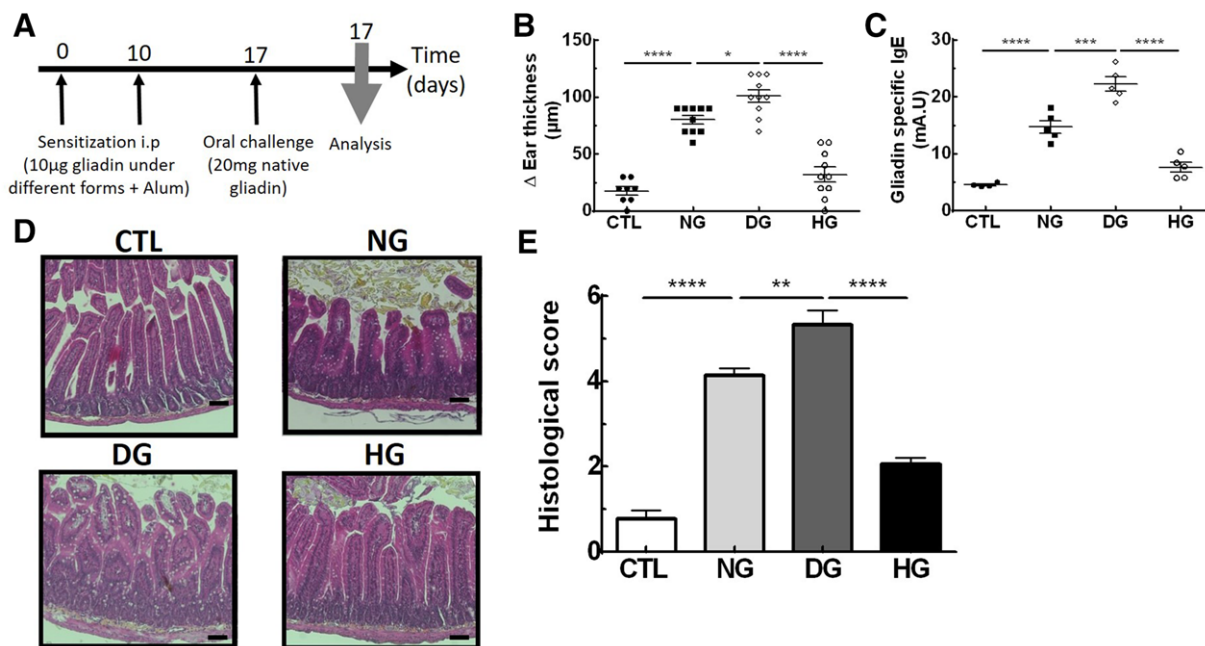


Figure 1. The deamidated forms of wheat gliadins amplify the allergic symptoms. A) Mice were sensitized by intra-peritoneal injection of either deamidated gliadins, NG, or HG and then challenged with NG by gavage. B) Measures of the ear thickness after the oral challenge. C) Measures of wheat-specific IgE in serum of the four groups of mice after the challenge. D) Hematoxylin-eosin stained jejunum sections from the CTL and mice sensitized to gliadins with NG, DG, or HG forms. Histological score calculated from jejunum sections according to inflammation, crypt damage, and ulceration in four groups of mice. Data represent the mean $\pm$ SEM ($n = 8-10$ animals per group); * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$. Scale bar = 100 μ m.

allergic to NG (4.13 vs 5.3, $p < 0.01$) (Figure 1E). Taken together, these results demonstrate that the sensitization and challenge with the deamidated form of gliadins strengthened the allergic response.

3.2. Deamidated Gliadins Induce Stronger Sensitization

To compare the development of the sensitization among the four groups, the concentrations of gliadin-specific IgE, IgG1, and IgA were determined after the first (data not shown) and the second sensitization (Figure 2). The levels of IgE, IgG1, and IgA after the first sensitization were close to the detection level and comparable for the four groups (data not shown). However, after the second sensitization, although unmodified gliadins (NG) did not induce a statistically significant higher IgE response compared to the HG or control group (CTL) (Figure 2A), the levels of specific IgG1 and IgA were increased (Figure 2B,C). In contrast, the highest level of specific IgE (15.1 vs 8.46, $p < 0.001$) and IgG1 (11.4 vs 20.2, $p < 0.001$) was obtained by immunization with deamidated gluten (Figure 2A,B) and was statistically higher than in mice allergic to native gluten. In contrast, the level of specific IgA was not different between NG and DG groups (0.54 vs 0.41).

3.3. Deamidated Gliadins Affect Gut Permeability

To assess whether Ig levels are associated with modification of intestinal barrier function, we evaluated intestinal transcellular

permeability using a Ussing chamber after the first and the second sensitizations (Figure 3). There was no difference between the four groups after the first sensitization (Figure 3A). The results showed an increase in HRP flux for the mice sensitized with the deamidated form of gliadins after the second sensitization compared to the mice sensitized with native or hydrolyzed forms (65.89 vs 11.17; $p > 0.05$ and 5.32 $p < 0.001$, respectively) (Figure 3B). These results suggest an increase of the intestinal epithelial layer permeability in an earlier manner with the deamidated form of gliadins than with the native form. To investigate a possible explanation of gut permeability modification, we measured relative expression of tight junction (TJ) proteins (Figure 3C): the junction adhesion molecule (JAM-A) involved in the regulation and maintenance of the TJ barrier,^[21] the ZO proteins (ZO-1) which are required to link occludin to the actin cytoskeleton,^[22] the occludin which has a crucial role in the TJ structure,^[23] and the cingulin which is a protein localized in the cytoplasmic region of TJ.^[24] Surprisingly, we did not find any significant modification of TJ protein expression except for ZO-1 which is significantly decreased in DG groups compared to controls (1.2 vs 0.71, $p < 0.05$) (Figure 3C).

3.4. Deamidated Gliadins Induce Dendritic Cells and Modify Th Responses

To characterize the immune response activated during sensitization, we analyzed cell infiltration in the MLNs after the second sensitization (Figure 4). In line with our previous results, the first sensitization was not effective at inducing an immune response

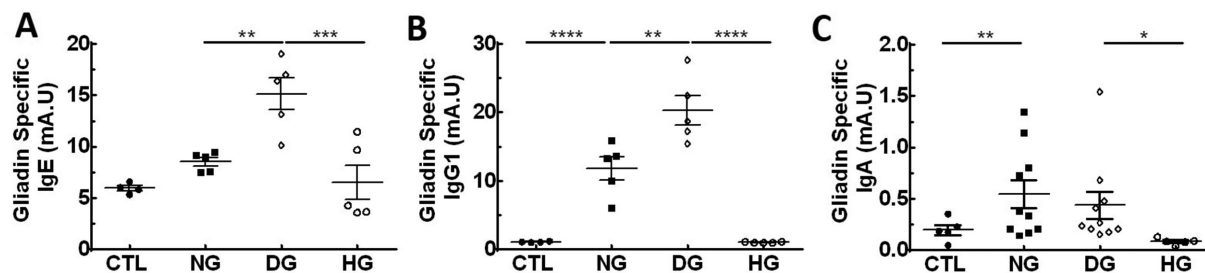


Figure 2. The deamidated forms of wheat gliadins amplify the allergic sensitization. Measures of wheat-specific A) IgE, B) IgG1, and C) IgA in serum from the four groups (CTL, black circle; NG, black square; DG, white diamond; and HG, white circle) of mice after two IP sensitizations. Data represent the mean $\pm$ SEM ($n = 5-10$ animals per group); * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

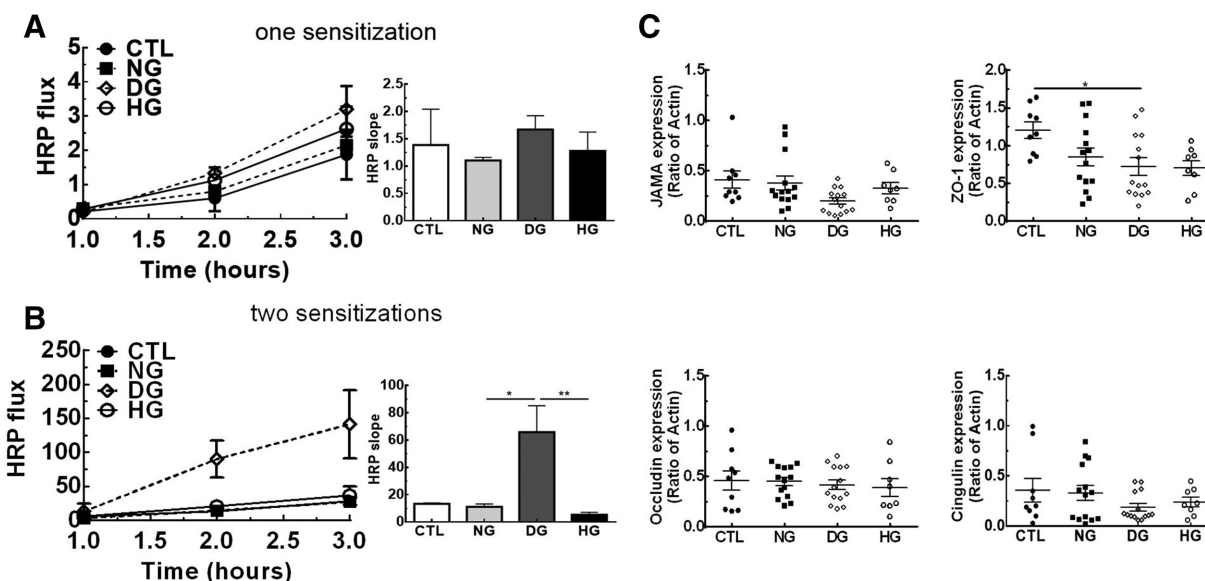


Figure 3. Sensitization with the deamidated form of gliadins increases intestinal permeability. Jejunum segments were extracted 1 h A) after the first sensitization and B) after the second sensitization, and the intestinal transcellular permeability to HRP was measured using a Ussing chamber over a 3 h period of time. C) Relative expression of tight junction protein was measured by western blot in the four groups of mice after the second sensitization. Proteins measured were JAMA (upper left panel), ZO-1 (upper right), occludin (lower left), and cingulin (lower right). Data represent the mean $\pm$ SEM ($n = 5-10$ animals per group); * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

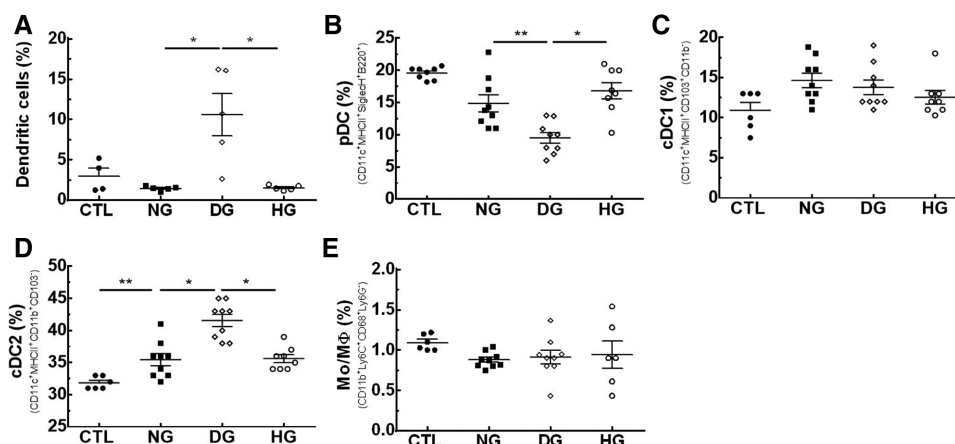


Figure 4. The deamidated form of the gliadins induces influx of cDC and defect in pDC. Frequency of A) DCs, B) pDC, C) cDC1, D) cDC2, and E) monocytes/macrophages in the MLNs after the second sensitization obtained by flow cytometry from the four groups of mice. Data represent the mean $\pm$ SEM ($n = 5-10$ animals per group); * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

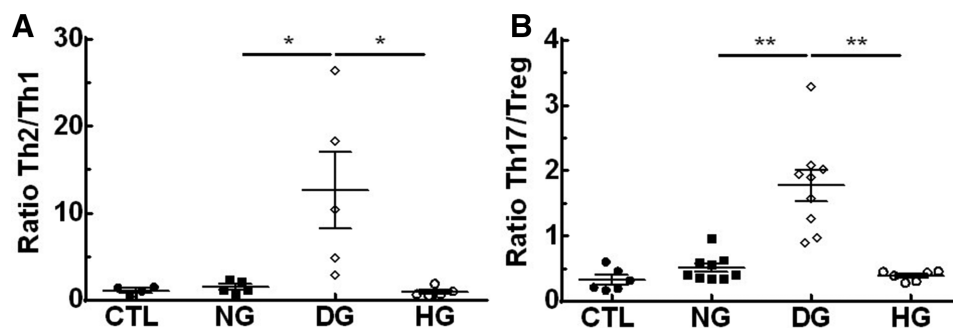


Figure 5. The deamidated form of the gliadins induces Th2 polarization and Th17 inflammation. A) Th1/Th2 and B) Th17/Treg ratios were measured in MLNs after the second sensitization obtained by flow cytometry from the four groups of mice. Th1 ($CD3^+CD4^+IFN^+$), Th2 ($CD3^+CD4^+IL-4^+$), Th17 ($CD3^+CD4^+ROR\gamma^+IL-17^+$), and Treg ($CD3^+CD4^+CD25^{high}FoxP3^+$) were measured after in vitro stimulation. Data represent the mean $\pm$ SEM ($n = 5$ –10 animals per group); * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

as there was no increase in dendritic cell frequency in MLNs (data not shown). In contrast, after the second sensitization with DG, an increase of two- to threefold was observed in the frequency of DCs in MLNs compared to native or hydrolyzed gliadin-sensitized mice (Figure 4A). The NG and HG groups did not show an increase in dendritic cell frequency compared to control mice. Transcriptomic, phenotypic, and functional analyses indicate that human and murine DCs can be divided into three subsets: two subsets of conventional DCs (cDC) called cDC1 and cDC2, and a third lineage of plasmacytoid DCs (pDCs). Each subset is unique and has distinct functional specificities to control T-cell responses. To examine which sub-population of DCs, we determined frequency of each population of DC. We showed that among DC, pDCs were significantly decreased in DG sensitized mice compared to HG or NG sensitized mice (Figure 4B). No difference was observed between HG- and NG-sensitized mice compared with control mice (Figure 4B). In contrast, frequency of cDC1 did not differ among the different groups of mice (Figure 4C). To the opposite, we observed an increase of cDC2 frequency in DG sensitized mice compared with HG or NG sensitized mice (Figure 4C). Finally, the ratio of monocytes/macrophages frequency did not reveal any difference between sensitized and control mice (Figure 4E). To determine whether this increase in cDC2 DCs is associated with lymphocyte differentiation, we measured the Th1/Th2 and the Th17/Treg ratio as they are known to be crucial factors in allergies (Figure 5). As such, after the first sensitization, the ratio between $CD3^+CD4^+IL-4^+$ Th2 cells and $CD3^+CD4^+IFN^+$ Th1 as well as $CD3^+CD4^+IL-17^+$ Th17 cells and $CD3^+CD4^+CD25^{high}FoxP3^+$ Treg cells were comparable among the four groups, demonstrating that one sensitization is not sufficient to induce immune response (data not shown). In contrast, after the second sensitization, the ratios of Th2/Th1 and Th17/Treg were largely increased in mice sensitized with deamidated gliadins compared to control mice. Moreover, the Th2/Th1 ratio remains unchanged in native and hydrolyzed gliadin-sensitized mice compared to controls (Figure 5A). To the opposite, Th17/Treg ratio was significantly increased in DG sensitized mice compared with NG or HG sensitized mice (Figure 5B). Overall, these results support that the deamidated form of gliadins induces an earlier allergic immune response in mice than native or hydrolyzed forms of gliadins.

4. Discussion

Because of recent reports concerning several cases of allergic reactions to modified glutens,^[25] the aim of our study was to investigate the allergenic potential of a gliadin fraction modified by deamidation or enzymatic hydrolysis compared with the native gliadin fraction. To examine the impact of gliadin deamidation on the sensitization and the elicitation phases of an allergic response, we used an intra-peritoneal (IP)-sensitized and an orally challenged Balb/c mouse model in which several immunological parameters have been measured.

The results of the present study demonstrated that deamidated gliadins induce a stronger allergic reaction than NG. First, DG allergic mice displayed higher ear swelling and higher concentrations of gliadin-specific IgE than NG-allergic mice or HG-allergic mice. Moreover, DG-allergic mice display an aggravation of intestinal damage after challenge compared with NG-allergic mice. Our results are consistent with the observations in humans. In fact, the symptoms of patients allergic to deamidated wheat proteins (anaphylactic shock, exercise-induced anaphylaxis (EIA), urticaria) are often more severe than symptoms of patients allergic to wheat (more often gut irritation, allergic rhinitis, asthma, and atopic eczema/dermatitis syndrome (AEDS) than EIA and anaphylactic shock).^[25,26] Our results demonstrate that acid hydrolysis or deamidation induced by industrial processes provokes an increase in allergic severity potential, as it has been suggested for peanut allergens.^[27] For peanuts, the allergic potential modification might be linked to protein structure. In contrast, strong enzymatic hydrolysis by Alcalase decreases the allergic potential of gliadins. This finding is in line with a study demonstrating that among several enzymes, Alcalase is stronger at modifying structure.^[28]

To understand how deamidation could enhance allergic potential of gliadins, we chose to examine Ig production, gut permeability, and immune response development during sensitization. We observed that one sensitization did not induce any change in these parameters. A second sensitization is sufficient to induce gliadin-specific IgE, IgG1, IgA, and an increase in gut permeability in the case of DG but to a lesser extent for NG or HG. Moreover, we also observed mounting of immune response characterized by an influx of DCs and a Th2/Th17 bias in a stronger manner for DG than NG or HG. Among DCs, pDCs are the

one mostly decreased whereas cDC2 is increased. These results demonstrate the importance of pDC in tolerance establishment by inducing Treg cells, as shown in ref. 29. Therefore, deamidation of gliadin induces a modification in pDC influx and consequently a stronger sensitization potential. This is associated with a stronger and a faster presence of cDC2 known for its ability to prime naive T cells and to generate various effector T (Teff) cell phenotypes because of their prominent expression of MHC class II and costimulatory molecules.^[30] These earlier allergic sensitization signs observed for DG might be related to the epitope structure of gliadins. Certain tandem sequence motifs that have glutamine residues in wheat gliadins have been reported to constitute the primary structure of IgE-binding epitopes.^[31,32] During deamidation, these epitopes are modified to create neo-epitopes. Deamidated gliadins have been shown to react with the sera of patients suffering from wheat allergy to some extent.^[25] These findings indicate that sensitization with deamidated forms of gliadins induces IgE directed to both specific and native epitopes, probably because of the exposure of the inner epitope structure to the surface during acidic hydrolysis and enzymatic reaction as we previously suggested.^[11] This finding demonstrates that deamidation of gliadins influences both the allergic sensitization potential and the triggering ability. In fact, deamidated gliadins display a reduction in triggering allergic reaction in mice sensitized with native gliadin.^[33] This might be linked to the fact that sensitization with native gliadin induces the production of native gliadin-specific IgE which is less able to bind deamidated gliadin during allergic reaction. In contrast, deamidated gliadins show stronger sensitizing potential compared with native gluten in a model of skin sensitized food allergy.^[34] Results demonstrate that the stronger sensitizing potential of deamidated gliadins is linked to an earlier attraction of DCs (mainly cDC2) associated with a lack of pDC to mesenteric lymph which in turn induces an earlier Th2/Th17 response. Under normal conditions, the Th1/Th2 cells are in a relatively balanced state in mice.^[35] When body dysfunctions such as anaphylaxis occur, they are characterized by a shift in the balance to Th2 cells, thereby resulting in a series of symptoms, including ear swelling, increased gut permeability, and mast cell degranulation. Moreover, a decrease in Treg cells and an inflammation link to Th17 cells are also observed and participate to inflammation and gut permeability in allergy. The classical paradigm of inflammatory pathogenesis involving exposure to environmental triggers has been challenged recently by the addition of a third element, the loss of intestinal barrier function. The loss of intestinal barrier function secondary to the activation of the zonulin pathway by food-derived environmental triggers or changes in gut microbiota seem to be key ingredients involved in the pathogenesis of food allergy^[21] but seems to be involved at a later stage, like after a third sensitization^[11] as no differences in TJ expression were observed except for ZO1. All of these symptoms were observed in Balb/c mice after oral provocation with gliadins in the present study. Our results were also consistent with the observations of previous studies,^[36,37] verifying that industrial processing affects the allergenicity of allergens in general. This study focused on the potential for industrial processes to modify major wheat allergens. Alcalase hydrolysis was found to be very effective at reducing the allergenic potential of gliadins. The acid hydrolysis inducing deamidation produced greater allergenic potential. In summary, our findings show that industrial processes could

modify the allergenic potential of gliadins by worsening or reducing its sensitizing capacity depending on the procedure. Our data also highlight that deamidation is sufficient for activating key immune pathways necessary for sensitizing mice for immediate hypersensitivity reactions. The data demonstrated the potential risk of partial hydrolysis of food proteins, in which hydrolyzed proteins may exhibit increased allergenic potency. Additionally, this experimental model using Balb/c mice may be very useful for further basic and applied studies of the sensitizing potential of various hydrolyzed proteins. The high incidence of allergies in the worldwide population presents challenges given the negative impact of a strictly gluten-free diet on the patients' perceived quality of life. A lifelong gluten-free diet is not easy to maintain since gluten is the most common ingredient in the human diet. Multidisciplinary research efforts are currently being carried out in several directions to find new treatment strategies in order to reduce gluten toxicity. The use of protease technologies currently represents a promising strategy.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

allergy, gliadins, mouse, permeability, Th2 cells

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