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Sensitization to Gliadin Induces Moderate Enteropathy and Insulitis in Nonobese Diabetic-DQ8 Mice

Heather J. Galipeau,* Nestor E. Rulli,† Jennifer Jury,* Xianxi Huang,* Romina Araya,‡ Joseph A. Murray,§ Chella S. David,§ Fernando G. Chirdo,† Kathy D. McCoy,*† and Elena F. Verdu*†

Celiac disease (CD) is frequently diagnosed in patients with type 1 diabetes (T1D), and T1D patients can exhibit Abs against tissue transglutaminase, the auto-antigen in CD. Thus, gliadin, the trigger in CD, has been suggested to have a role in T1D pathogenesis. The objective of this study was to investigate whether gliadin contributes to enteropathy and insulitis in NOD-DQ8 mice, an animal model that does not spontaneously develop T1D. Gliadin-sensitized NOD-DQ8 mice developed moderate enteropathy, intraepithelial lymphocytosis, and barrier dysfunction, but not insulitis. Administration of anti-CD25 mAbs before gliadin-sensitization induced partial depletion of CD25+Foxp3+ T cells and led to severe insulitis, but did not exacerbate mucosal dysfunction. CD4+ T cells isolated from pancreatic lymph nodes of mice that developed insulitis showed increased proliferation and proinflammatory cytokines after incubation with gliadin but not with BSA. CD4+ T cells isolated from nonsensitized controls did not respond to gliadin or BSA. In conclusion, gliadin sensitization induced moderate enteropathy in NOD-DQ8 mice. However, insulitis development required gliadin-sensitization and partial systemic depletion of CD25+Foxp3+ T cells. This humanized murine model provides a mechanistic link to explain how the mucosal intolerance to a dietary protein can lead to insulitis in the presence of partial regulatory T cell deficiency. The Journal of Immunology, 2011, 187: 4338–4346.

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*Farncombe Family Digestive Health Research Institute, McMaster University Medical Centre, Hamilton, Ontario L8N 3Z5, Canada; †Laboratorio de Investigacion en el Sistema Inmune, Departamento de Ciencias Biologicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentina; ‡Department of Internal Medicine, Mayo Clinic College of Medicine, Rochester, MN 55905; and §Department of Immunology, Mayo Clinic College of Medicine, Rochester, MN 55905

†Current address: Department of Clinical Research, University of Bern, Bern, Switzerland.

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Address correspondence to Dr. Elena F. Verdu, Department of Medicine, McMaster University, 1200 Main Street West Hamilton, ON L8N 3Z5, Canada. E-mail address: verdu@mcmaster.ca

The online version of this article contains supplemental material.

Abbreviations used in this article: CD, celiac disease; IEL, intraepithelial lymphocyte; MLN, mesenteric lymph node; NOR, nonobese resistant; PBS-T, PBS-0.05% Tween-20; PLN, pancreatic lymph node; PT-gliadin, peptic-tryptic digest of gliadin; T1D, type 1 diabetes; Treg, regulatory T cell; tTG, tissue transglutaminase.
Materials and Methods

Transgenic male mice that express HLA-DQ8 in an endogenous MHC class II-deficient background were backcrossed to NOD mice for 10 generations and intercrossed to produce congenic NOD ABβ DQ8 mice (33). Eight- to 10-wk-old male mice were used for experiments. Mice were weaned and maintained on a low-fat (4.4%), gluten-free diet, purchased from Harlan Laboratories and bred in a conventional, specific pathogen-free colony at McMaster University. Fourteen-week-old male NOD mice, 6-wk-old NOD mice, and 14-wk-old nonobese resistant (NOR) mice (obtained from Dr. J. Danska, Hospital for Sick Children, Toronto, ON, Canada) were used in additional experiments as positive and negative controls, respectively, for the development and evaluation of insulitis. Glycemic status was monitored weekly using a glucometer (Abbott Diabetes Care) and was determined at the time of sacrifice using a Roche modular instrument. All experiments were conducted with approval from the McMaster University Animal Care Committee.

Anti-CD25 Ab treatment

Prior to gliadin sensitization, mice received two i.p. injections of monoclonal anti-CD25 Abs (PC61, 250 μg; Leinco Technologies, St. Louis, MO), 1 wk apart. This procedure induces a partial depletion of CD4+ CD25+ Foxp3+ T cells and induces an immune dysregulation. Control groups were pretreated with two i.p. injections of PBS (250 μl) 1 wk apart.

Gliadin sensitization

One week after anti-CD25 Ab treatment, mice were sensitized with a peptic-digesty extract of gliadin (PT-gliadin). PT-gliadin was prepared as described previously (35). Gliadin (Sigma-Aldrich) was dissolved in endotoxin-free 0.2 N HCl for 2 h in a 37°C water bath with 1 g pepsin (Sigma-Aldrich). After 2 h of digestion, the pH was adjusted to 7.4 using endotoxin-free 2 M NaOH. Trypsin (Sigma-Aldrich) was added, the solution was boiled vigorously for 30 min, and the PT-gliadin was stored at −20°C. To sensitize the mice, they were gavaged with 500 μg PT-gliadin plus 25 μg choleratoxin (Sigma-Aldrich) once per week for 3 wk. Gliadin-sensitized mice were switched to a gluten-containing diet and an immune dysregulation. Control groups were pretreated with two i.p. injections of PBS (250 μl) 1 wk apart.

Glucose tolerance test

To determine impaired glucose tolerance, mice were fasted for 6 h. Mice were then injected with glucose (Sigma-Aldrich) i.p. at a dose of 1 g/kg. Venous plasma glucose was checked prior to glucose injection, and at 20, 40, 60, 90, and 120 min after glucose injection (36). Blood glucose levels were tested using a glucometer (Abbott Diabetes Care). Glucose tolerance tests were conducted after the third gliadin-sensitization.

FACS analysis

One week after the second anti-CD25 Ab injection, cell suspensions of spleen, mesenteric lymph nodes (MLNs) and PNs were prepared in RPMI 1640 (1% Penstrept, 10% FCS, 2 mM L-glutamine) by passing organs through a 100-μm nylon mesh screen to dissociate the cells. Suspensions were depleted of RBCs by lysis and resuspended in FACS buffer (PBS containing 0.1% azide and 2% BSA). Cells were stained with fluorescein-labeled cell-surface Abs including CD4-allophycocyanin (RM4-5), CD8a-PerCP (53-6.7), and CD25-PE (7D4) for 30 min at 4°C (BD Biosciences-Pharminagen). For intracellular staining, cells were permeabilized using the Fix/Perm 3 staining buffer set (eBioscience) and incubated with FITC-conjugated Abs toward Foxp3 (FJK-16s; eBioscience) for 90 min at 4°C. Stained cells were acquired using the LSR II (BD Biosciences) and analyzed with FlowJo software (TreeStar).
In vitro proliferation assay and cytokine analysis

Cell suspensions of PLNs were prepared in RPMI 1640 (1% penicillin/streptomycin, 10% FCS, 2 mM L-glutamine) by passing organs through a 100-μm nylon mesh screen to dissociate cells. CD4+ T cells were isolated from PLNs through negative selection (EasySep Mouse CD4+ T Cell Enrichment Kit, Stemcell). Cell yield was assessed by FACs analysis, with an enriched population of 89–96% CD4+ T cells. Isolated CD4+ T cells were labeled with CFSE as previously described and washed three times with RPMI 1640 (44). Splenocytes were treated with mitomycin C (Sigma-Aldrich), and dendritic cells were isolated through positive selection (EasySep Mouse CD11c Selection Kit, Stemcell). Cell yield was assessed by FACS analysis, with an enriched population of 89–96% CD4+ T cells. Isolated CD4+ T cells were labeled with CFSE as previously described and washed three times with RPMI 1640 (44).

Supernatants from cell cultures were collected at 48 and 96 h. The presence of proinflammatory cytokines in the supernatant was determined using a cytometric bead array inflammation kit (BD Biosciences) and analyzed using BD FACScArray Bioanalyzer System (BD Biosciences). The presence of TGF-β was determined by ELISA (R&D Systems).

Statistical analysis

Statistical analysis was performed with GraphPad Prism software. For more than two treatment groups, an ANOVA with a Bonferroni post hoc test for multiple comparisons was used. When two groups were compared, an unpaired t test was used; p < 0.05 was considered significant. Data are displayed as mean ± SEM.

Results

Gliadin-sensitized NOD-DQ8 mice developed barrier dysfunction and altered villus/crypt ratios

We have shown previously that HLA-DQ8 mice develop activation of the innate and adaptive arms of the immune system, and increased tissue conductance after gliadin sensitization (41, 43, 45). Despite this finding, gliadin-sensitized HLA-DQ8 mice develop only a mild enteropathy, characterized by increased IELs, similar to a Marsh I lesion in CD (46). To determine whether NOD-DQ8 mice develop gliadin-induced barrier dysfunction and enteropathy, mice were sensitized with a peptic-tryptic digested form of gliadin once per week for 3 wk. Cholera toxin was used as a mucosal adjuvant. A group of mice was pretreated with anti-CD25 mAbs to determine whether a preexisting immune dysregulation could exacerbate gliadin-induced mucosal dysfunction and enteropathy. Regulatory T cells (Tregs) are marked by the expression of CD4, CD25, and the transcription factor Foxp3. Therefore, we define a Treg as a CD4+CD25hiFoxp3hi T cell, and will refer to CD4+CD25hiFoxp3hi T cells as Tregs or CD4+CD25hiFoxp3+ Tregs hereafter. Furthermore, CD25 (IL-2Rα subunit) is essential for the generation, peripheral expansion, and maintenance of Tregs (47–52). Monoclonal anti-CD25 Abs have been used to deplete the Treg population (47, 49, 53). Therefore, NOD-DQ8 mice were treated with two i.p. doses of anti-CD25 mAbs, 1 wk apart, prior to gliadin sensitization. We used Ussing chambers to measure tissue conductance in sections of the small intestine. Enteropathy was evaluated by villus-to-crypt ratios and by immunostaining for CD3+ IELs (37, 41).

Gliadin-sensitized NOD-DQ8 mice had increased tissue conductance compared with untreated controls and to anti-CD25 treated mice (Fig. 1). No difference in conductance was seen between untreated controls and anti-CD25 mAb-treated plus BSA-sensitized mice. There was also no difference in tissue conductance between gliadin-sensitized mice and sensitized mice that were pretreated with anti-CD25 mAbs, suggesting that barrier dysfunction was gliadin-dependent and was not induced by anti-CD25 mAb treatment. Gliadin-sensitized mice developed lower villus-to-crypt ratios (Fig. 2) and increased IEL counts (Fig. 3), reminiscent of a Marsh II lesion, compared with untreated control mice, anti-CD25 mAb treated mice, and anti-CD25 mAb-treated plus BSA-sensitized mice. Previous Treg depletion did not further increase the severity of gliadin-induced enteropathy in NOD-DQ8 mice. These results suggest that, compared with HLA-DQ8 mice, DQ8 mice on the NOD background are more sensitive to gliadin-sensitized mice.
induced enteropathy, and partial depletion of Tregs has no evident role in the induction of enteropathy in the model.

**Gliadin-sensitized NOD-DQ8 mice develop anti-gliadin and anti-tTG Abs**

In contrast to anti-gliadin IgA and IgG Abs, IgA autoantibodies toward tTG are highly specific for CD (54). HLA-DQ8 mice develop anti-gliadin IgA after gliadin-sensitization (41, 45). We tested whether gliadin sensitization in NOD-DQ8 mice led to the production of Abs toward gliadin and tTG. Four of 11 gliadin-sensitized mice developed anti-gliadin IgA Abs in the serum and three of nine anti-CD25 plus gliadin-sensitized mice developed anti-gliadin IgG Abs. Anti-gliadin IgG Abs were not detected in the serum of nonsensitized mice (Fig. 4A). Anti-tTG IgA Abs were found in the serum of three gliadin-sensitized mice and two anti-CD25 mAb-treated plus gliadin-sensitized mice. No control, anti-CD25 mAb treated only, or BSA-sensitized mouse tested positive for anti-tTG Abs (Fig. 4B). The presence of anti-tTG Abs in a proportion of gliadin-sensitized mice is in accordance with the more moderate enteropathy observed in these mice compared with HLA-DQ8 mice (41).

**Anti-CD25 Ab treatment partially depleted CD4⁺CD25⁺Foxp3⁺ T cells**

To confirm that anti-CD25 mAb treatment depleted the regulatory population, NOD-DQ8 mice were treated with two i.p. doses of anti-CD25 mAbs, each 1 wk apart. Cells were collected 1 wk following the second Ab treatment, and the percentage of Tregs was determined. Similar to previous findings, we showed a partial depletion of CD4⁺CD25⁺Foxp3⁺ Tregs (Supplemental Fig. 1) (47, 49). Compared with PBS-treated controls, the CD25⁺Foxp3⁺ cells decreased from 10.57 to 2.32% of the total CD4⁺ T cell population in the spleen of anti-CD25 mAb-treated mice (Supplemental Fig. 1A, left panels). The remaining Foxp3⁺ cells expressed low levels of CD25 or no CD25. In addition, anti-CD25 mAb treatment resulted in the partial depletion of Tregs in the MLN and PLN. In the MLN, the CD25⁺Foxp3⁺ cells decreased from 11.54 to 3.27% of the total CD4⁺ T cell population (Supplemental Fig. 1A, center panels). In the PLN, the CD25⁺Foxp3⁺ cells decreased from 11.94 to 3.0% of the CD4⁺ T cell population (Supplemental Fig. 1A, right panels). Thus, anti-CD25 mAb treatment before gliadin sensitization led to a partial, but significant depletion of Tregs in the spleen, MLN, and PLN (Supplemental Fig. 1B).

**Partial Treg depletion and gliadin sensitization was associated with insulitis in NOD-DQ8 mice**

NOR and NOD mice were used as negative and positive controls, respectively, to validate the insulitis grading in NOD-DQ8 mice. At 6 wk of age, NOD mice had developed mild periinsulitis (Supplemental Fig. 2A, 2D), while severe insulitis developed at 14 wk of age (Supplemental Fig. 2B, 2D). NOR mice developed mild periinsulitis at 14 wk of age (Supplemental Fig. 2C, 2D), as previously reported (55). Intestinal tissue conductance was significantly higher in 14-wk-old NOD mice, but not in 6-wk-old NOD mice, compared with NOR controls. NOR mice displayed normal barrier function (Supplemental Fig. 3). Thus, although NOD mice displayed higher tissue conductance values at the preinsulitis stage, only at 14 wk of age (insulitis stage) did this difference become statistically significant.

We investigated whether gliadin-induced mucosal changes were linked to insulitis in NOD-DQ8 mice. Previous studies have shown that NOD-DQ8 mice on a gluten-containing diet develop a gliadin-dependent blistering of the skin, but do not develop diabetes (33). In addition, gliadin-sensitized HLA-DQ8 mice have shown
increased IL-10 production and increased recruitment of regulatory Foxp3+ cells within the lamina propria. It has been hypothesized that this regulatory immune response to gliadin may protect mice from overt autoimmunity (41, 45). Thus, we used anti-CD25 mAbs to partially deplete the Tregs, which we defined as CD4+CD25+ Foxp3+ T cells (47, 56) prior to gliadin sensitization. Pancreatic islets were analyzed for infiltration of immune cells 24 h after the final gavage. Untreated control mice had normal islets (Fig. 5A). Anti-CD25 treated mice also had normal islets with no significant infiltration (Fig. 5B). Similar to the findings of Marietta et al. (33), gliadin-sensitized mice did not develop severe insulitis (Fig. 5C). However, mice that were pretreated with anti-CD25 mAbs and subsequently sensitized with gliadin developed severe insulitis (Fig. 5E). The insulitis scores of anti-CD25 plus gliadin-sensitized mice were similar to those of 14-wk-old NOD mice (Supplemental Fig. 2). Treatment of anti-CD25 mAbs plus BSA sensitization did not lead to insulitis, suggesting that the response was not induced by an unrelated Ag and was gliadin-dependent (Fig. 5D). The insulitis scores of anti-CD25 mAb treated plus gliadin-sensitized mice were greater than those in untreated controls, anti-CD25 mAb treated mice, gliadin-sensitized, and to anti-CD25 mAb treated plus BSA-sensitized mice (Fig. 5F). The infiltrates were composed of CD3+ lymphocytes (Fig. 6). No differences were seen in blood glucose levels (data not shown) or glucose tolerance between groups (data not shown). These results suggest that NOD-DQ8 mice are susceptible to developing an inflammatory response in β-cell islets when systemic partial depletion of CD4+CD25+Foxp3+ cells is induced prior to gliadin sensitization.

Partial CD4+CD25+Foxp3+ cell depletion and gliadin sensitization did not lead to widespread autoimmunity

To determine whether inflammation was present in other organs, H&E-stained sections of the liver and lungs were examined for signs of infiltration and inflammation. There was no significant infiltration observed in the liver of nonsensitized controls, anti-CD25 mAb treated mice, gliadin-sensitized mice, anti-CD25 mAb treated plus BSA-sensitized mice, or in anti-CD25 mAb plus gliadin-sensitized mice (data not shown). Similarly, none of the groups exhibited inflammation within the lungs (data not shown). Thus, the infiltration observed in anti-CD25 mAb-treated plus gliadin-sensitized mice was restricted to the pancreas, indicating an absence of generalized autoimmunity.

T cells from PLNs of NOD-DQ8 mice that developed insulitis exhibit increased proliferation and proinflammatory cytokine production when incubated with gliadin

HLA-DQ8 mice have shown increased proliferative responses toward gliadin peptides in both the spleen and MLN (45, 57). Evidence suggests that the gut and the pancreas are immunologically linked. In NOD mice, islet-infiltrating lymphocytes express the α4β7 integrin, a gut-homing receptor (58). The ligand for α4β7, MAdCAM-1, is expressed within the pancreas and is
upregulated during insulitis (59). These findings suggest that lymphocytes displaying gut homing markers are able to circulate between the gut and the pancreas, and that immune responses to dietary Ags may modulate insulitis. We therefore determined whether cells in the PLNs of gliadin-sensitized NOD-DQ8 mice responded to gliadin by measuring proliferation and cytokine production. CD4+ T cells were isolated from the PLNs of control mice and anti-CD25 mAb plus gliadin-sensitized mice and incubated with gliadin, BSA, or media alone. In anti-CD25 mAb plus gliadin-sensitized mice, gliadin stimulation led to increased proliferation compared with BSA and media alone. In control mice, no increase in proliferation was observed in gliadin-stimulated cultures (Fig. 7A, 7B). Compared with untreated controls, gliadin stimulation increased proliferation in anti-CD25 plus gliadin-sensitized mice. Cytokine production was measured in the PLN cell culture supernatants. There was a significant increase in production of the proinflammatory cytokine TNF-α in the cell cultures from mice that developed insulitis and were incubated with gliadin compared with BSA (Fig. 7C). In cultures from mice that developed insulitis and were cultured with media or BSA, the production of MCP-1 and IL-6 was below the limit of detection, whereas incubation with gliadin resulted in detectable levels of both MCP-1 and IL-6 in some of the mice (Fig. 7D, 7E). No differences were found in IL-10 or TGF-β production (data not shown). In PLN cell cultures from control mice, all cytokine levels were below the limit of detection (data not shown). These results suggest that gliadin-specific T-cells are found within the PLNs of mice that develop insulitis and that these cells produce a Th1 cytokine response.

Discussion

The main objective of this study was to investigate the role of gliadin in the development of enteropathy and insulitis in NOD-DQ8 mice. T cells in NOD-DQ8 mice are selected in the thymus in a DQ8-restricted manner. The presence of the DQ8 gene ensures efficient gliadin Ag presentation by APCs to CD4+ T cells (45, 57, 60). We found that gliadin sensitization in NOD-DQ8 mice induced moderate enteropathy, characterized by decreased villus-to-crypt ratios and increased IEL counts in the proximal small intestine. Similar to other DQ8 transgenic mouse models, NOD-DQ8 mice developed gliadin-induced intestinal barrier dysfunction. These changes were induced by gliadin, as the immune and functional changes were not observed after sensitization with an unrelated Ag—BSA. Development of severe insulitis, however, required partial depletion of CD25+Foxp3+ T cells prior to gliadin sensitization. T cells isolated from the PLNs of mice that developed insulitis exhibited enhanced proinflammatory cytokine production and proliferation when cultured with gliadin.
compared with culture with BSA or media alone. CD4+ T cells isolated from the PLNs of control mice did not respond to gliadin or BSA. These data suggest that a combination of mucosal damage and breakdown in tolerance to gliadin led to insulitis in NOD-DQ8 mice.

There is mounting evidence that a defect in the intestinal barrier can promote T1D (61, 62). Several animal models of T1D have demonstrated that increased intestinal permeability can be detected prior to the clinical onset of diabetes (63–65). Barrier dysfunction has also been observed in patients with T1D and in their relatives (66, 67). A recent study in NOD mice has shown that infection with *Cockroach rodentium* resulted in increased intestinal permeability and accelerated onset of insulitis and diabetes (68). The results suggest an adjuvant role of intestinal barrier dysfunction in diabetes development. In our study, 14-wk-old NOD mice used as positive controls for insulitis exhibited barrier dysfunction and severe insulitis. Because gliadin is known to affect intestinal permeability in animal models of gluten sensitivity and in humans with CD (41, 65, 69–71), we investigated whether NOD-DQ8 mice developed gliadin-induced barrier dysfunction and insulitis. It has been suggested that the patients with CD and the greatest risk of developing extraintestinal autoimmunity are those who have had a longer duration of gluten exposure (72). Studies in animal models and humans have shown a reduction in T1D risk following implementation of a gluten-free diet (29, 73, 74). We found that gliadin sensitization led to increased tissue conductance and an enteropathy, characterized by decreased villus-to-crypt ratios and increased IELs, that is reminiscent of moderate gluten sensitive enteropathy (75). In addition, a proportion of gliadin-sensitized mice developed both anti-gliadin IgG and anti-iTG IgA Abs. Similarly, a recent article using DQ8 transgenic mice that overexpress IL-15 in the lamina propria found that mice fed gliadin developed anti-gliadin and anti-iTG Abs, intraepithelial lymphocytosis, and IFN-γ-producing T cells, but lacked overt villous atrophy (76). Innate immune responses toward gliadin can directly cause mucosal damage, a process that involves production of IL-15. iTg also plays a key role in modifying the anti-gliadin immune response in CD. Whether these key innate immune responses have critical roles in the development of mucosal damage in NOD-DQ8 still needs to be elucidated. Despite these mucosal abnormalities in gliadin-sensitized NOD-DQ8 mice, insulitis did not develop. These findings suggest that loss of barrier function and moderate enteropathy induced by gliadin are not sufficient, on their own, to induce insulitis.

Recent studies have shown that inducible Tregs have a crucial role in maintaining oral tolerance (77). In the MLNs, IL-2 and TGF-β are required to induce production of CD4+CD25+Foxp3+ inducible Tregs from naive CD4+CD25− T cells (78). Migration to the gut and expansion of these cells in the lamina propria by intestinal dendritic cells is crucial for the generation of oral tolerance (77, 78). In accordance with previous results using this technique, we showed that treatment with anti-CD25 mAbs led to partial depletion of Foxp3+ Tregs (47, 49, 52). Importantly, this includes the inducible Treg population, which is critical for controlling the immune response in the intestinal mucosa against oral Ags (52, 77). The partial depletion was likely sufficient to disrupt the immunologic balance between proinflammatory and regulatory mediators in NOD-DQ8 mice because insulitis developed after sensitization with the relevant Ag in this model—gliadin. However, anti-CD25 mAb-treated plus gliadin-sensitized mice did not develop hyperglycemia or impaired glucose tolerance and were not, therefore, clinically diabetic at the time they were analyzed. Similarly, a study using anti-CD25 mAbs demonstrated severe autoimmune gastritis only after administration of autoimmune gastritis target Ag plus anti-CD25 mAb treatment (53). Although insulitis was evident in anti-CD25 mAb plus gliadin-sensitized mice, a longer follow-up time after gliadin sensitization may be needed for development of hyperglycemia and progression to diabetes. However, the degree of insulitis in anti-CD25 plus gliadin-sensitized mice was similar to the insulitis seen in 14-wk-old NOD mice in our study and reported elsewhere (79). Importantly, several studies suggest that a proinflammatory environment within the pancreatic islets can promote the development of diabetes. NOD-DQ8 mice have been shown to develop diabetes if TNF-α is overexpressed in β islets (34). Other studies have shown that the expression of the costimulatory molecule, B7-1, or IFN-γ within the islets can promote diabetes development, even in a diabetes-resistant background (80, 81). Our results indicate that partial CD25+Foxp3+ T cell depletion prior to gliadin sensitization was associated with islet infiltration in NOD-DQ8 mice. Extra-intestinal inflammation in gliadin-sensitized NOD-DQ8 mice with prior CD25+Foxp3+ cell depletion was localized to the pancreas, as liver and lung tissues were normal.

CD4+ T cells isolated from the PLNs of mice that developed insulitis responded to gliadin, and not to BSA. In NOD mice and patients with T1D, diabetogenic T cells within the islets display the gut homing marker α4β7 (82). Furthermore, the ligand for the α4β7 integrin, MadCAM-1, is expressed on the endothelium in the pancreas and is upregulated during insulitis (83). It has been shown that T cells isolated from the MLNs of 3-wk-old NOD mice are able to induce the development of diabetes in NOD.SCID mice more efficiently than lymphocytes isolated from peripheral lymph nodes, pancreatic lymph nodes, and the spleen (84). This finding suggests the initial priming of diabetogenic T cells may take place in the gut and that activated T cells can home to the pancreas to cause insulitis (84). In our model, oral sensitization to gliadin in mice with partially depleted Tregs led to the production of gliadin-responding T cells in the PLN. This gliadin-associated insulitis could be permissive of T1D development in a genetically predisposed host. Gliadin predominantly activates innate mechanisms in animal models, but the adaptive immune system has also been shown to be activated in transgenic HLA DQ8 mice (41, 45). We demonstrate the presence of gliadin-responsive T cells in the PLNs of NOD-DQ8 mice, providing and experimental setting where both innate and adaptive immune mechanisms can be studied within and outside the gut.

The current prevalence of T1D is estimated to double by 2020 in children younger than 5 y, and ~10% of children with T1D have documented CD (10, 85). CD is common worldwide, with a dramatic increase in prevalence from just 0.2–0.9% during the last five decades (6). With 95% of CD cases estimated to be currently undiagnosed in North America, and the increasing incidence of both diseases, the potential effects of gliadin intolerance on T1D are alarming. Diabetes imposes a substantial cost burden on society, in part because of its complications and comorbidities (86, 87). Our results provide a model to elucidate the mechanisms through which a common dietary intolerance may increase T1D risk and test innovative dietary approaches to prevent and improve metabolic control in T1D (88).

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Disclosures

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