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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

A B-Cell Gene Signature Correlates With the Extent of Gluten-Induced Intestinal Injury in Celiac Disease

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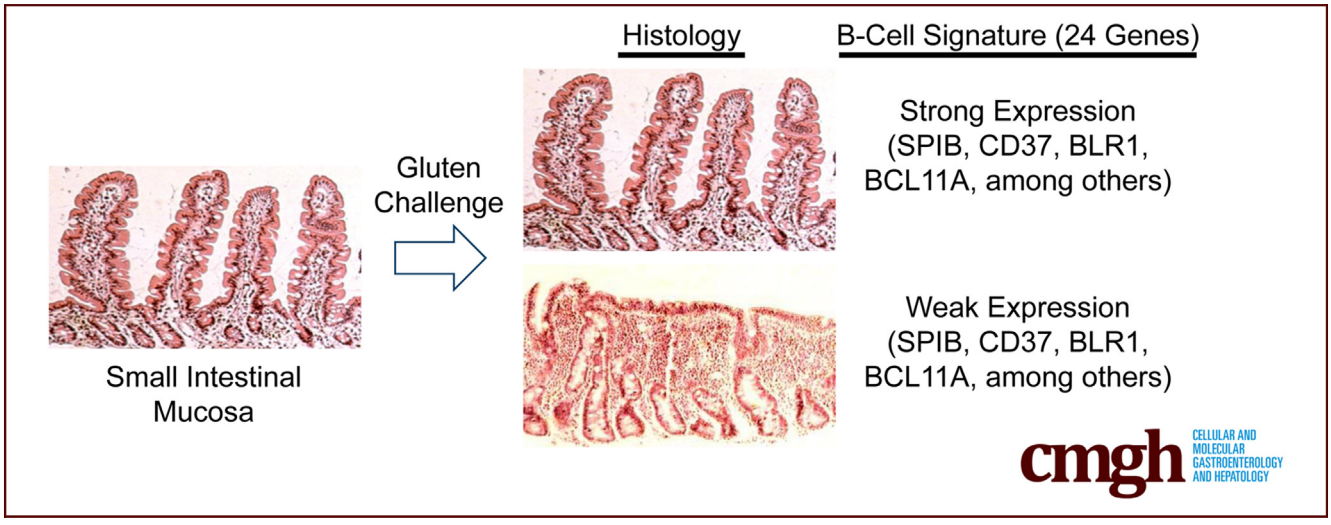

SUMMARY

A gluten challenge in celiac patients provides a unique opportunity to study the immunology associated with the transition from relative health to autoimmunity. This study showed that a B-cell population in peripheral blood correlated inversely with gluten-dependent small intestinal lesions, implicating a protective mechanism.

BACKGROUND & AIMS: Celiac disease (CeD) provides an opportunity to study autoimmunity and the transition in immune cells as dietary gluten induces small intestinal lesions.

METHODS: Seventy-three celiac disease patients on a long-term, gluten-free diet ingested a known amount of gluten daily for 6 weeks. A peripheral blood sample and intestinal biopsy specimens were taken before and 6 weeks after initiating the gluten challenge. Biopsy results were reported on a continuous numeric scale that measured the villus-height–to–crypt-depth ratio to quantify gluten-induced intestinal injury. Pooled B and T cells were isolated from whole blood, and RNA was analyzed by DNA microarray looking for changes in peripheral B- and T-cell gene expression that correlated with changes in villus height to crypt depth, as patients maintained a relatively healthy intestinal mucosa or deteriorated in the face of a gluten challenge.

RESULTS: Gluten-dependent intestinal damage from baseline to 6 weeks varied widely across all patients, ranging from no change to extensive damage. Genes differentially expressed in B cells correlated strongly with the extent of intestinal damage. A relative increase in B-cell gene expression correlated with a lack of sensitivity to gluten whereas their relative decrease correlated with gluten-induced mucosal injury. A core B-cell gene module, representing a subset of B-cell genes analyzed, accounted for the correlation with intestinal injury.

CONCLUSIONS: Genes comprising the core B-cell module showed a net increase in expression from baseline to 6 weeks in patients with little to no intestinal damage, suggesting that these individuals may have mounted a B-cell immune response to maintain mucosal homeostasis and circumvent

inflammation. DNA microarray data were deposited at the GEO repository (accession number: GSE87629; available: https://www.ncbi.nlm.nih.gov/geo/). *(Cell Mol Gastroenterol Hepatol 2017;4:1–17; http://dx.doi.org/10.1016/j.jcmgh.2017.01.011)*

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Human beings ingest a wide variety of food proteins in the diet that are considered foreign with respect to the immune system. Immune cells in the small intestine survey the contents in the intestinal environment looking for pathogens. Oral tolerance is a mechanism that balances the need to promote tolerance to orally administered, foreign, yet harmless, food proteins with the need to provide a host defense against harmful pathogens in the intestine.[1] Although not well understood, oral tolerance to a food protein is an active immune response whose function is to suppress inflammatory immune responses to the same food protein when presented to the immune system for a second time. In celiac disease (CeD), a lack of oral tolerance develops to a family of cereal proteins collectively referred to as gluten,[2] resulting in a pathogenic and inflammatory immune response.

The small intestinal mucosa consists of an epithelium and its underlying structures, which are immediately adjacent to the intestinal lumen and in contact with digested food. To increase surface area for nutrient absorption, the mucosa projects finger-like extensions called villi into the lumen of the gut. At the base of the villi are proliferative crypts. In patients with CeD, gluten ingestion results in blunting of the villi and hypertrophy or elongation of the crypts. The ratio of the height of the villi (Vh) to the depth of the crypt (Cd), expressed as Vh:Cd, has been used to quantify the extent of intestinal damage in CeD.[3–8] In severe cases, villi shrink completely with extensive crypt elongation, resulting in a flat mucosa and a Vh:Cd measurement approaching zero.

HLA-DQ is an important genetic factor that predisposes individuals to CeD and type 1 diabetes.[9,10] A total of 5%–10% of individuals with type 1 diabetes develop CeD,[11] which is significantly higher than the chance of developing CeD in the overall Caucasian population, estimated to be 1%.[12] Most CeD patients (90%) express HLA-DQ2.5, whereas the remainder express HLA-DQ2.2 or HLA-DQ8.[13] Gluten peptides that are deamidated by the self-protein transglutaminase 2 (TG2) bind strongly to HLA-DQ and the resulting complex is presented to HLA-DQ–restricted CD4+ T cells,[14] resulting in a T-cell response to deamidated gluten.[15] In addition to the T-cell response, gluten-dependent, disease-specific B cells appear early during disease pathogenesis. They precede gut damage, often are predictive of impending disease,[16–20] and produce antibodies specific for deamidated gluten and TG2.[21] It is unclear whether gluten-dependent auto-antibodies against TG2 contribute to the disease and there is little evidence that T cells with specificity for self-antigens drive the disease.

The B cell recognizes a specific protein antigen, such as gluten or TG2, through direct interactions with its B-cell receptor containing a membrane-bound immunoglobulin.[22] The immunoglobulin determines antigen specificity and is unique for each B-cell clone. B-cell receptor signaling contributes strongly to B-cell proliferation and differentiation. B cells can be antigen-presenting cells[23] and have the ability to express HLA-DQ2 or HLA-DQ8. Because gluten peptides are excellent substrates for TG2 and the two proteins form a transient covalent complex, the possibility exists that a deamidated gluten- or TG2-specific B cell binds the complex through the B-cell receptor, internalizes, and then presents gluten on HLA-DQ2 or HLA-DQ8 at the B cell surface to a gluten-specific HLA-DQ2– or HLA-DQ8–restricted CD4+ T cell.[15,24] In this scenario, the resulting B cell is a functional antigen-presenting cell whose cell phenotype, as determined by B-cell–receptor signaling and direct B- and T-cell co-stimulatory interactions, ultimately may be under the control of gluten.

An inflammatory, gluten-induced immune response in the gut may propagate systemically in peripheral blood. The plausible trafficking of B and T cells and anti-TG2 to sites beyond the gut may help to explain several systemic clinical manifestations of CeD,[25] including dermatitis herpetiformis, a gluten-dependent blistering skin condition. CeD also may manifest as a bone disease,[26] in the central nervous system as ataxia and brain atrophy,[27] or as an isolated subclinical[28] or severe liver disease.[29] Evidence indicates that immune cells migrate to and from the gut in peripheral blood. For example, disease-specific T cells expressing the gut-homing $\beta 7$ integrin migrate transiently to the periphery upon gluten challenge in CeD patients; these T cells are inflammatory in nature.[30,31] The peripheral blood therefore may be a good source to obtain biomarkers of the disease.

It is not clear how gluten interacts with mechanisms of peripheral immune tolerance and whether B or T cells are responsible for disrupting the tolerogenic environment of the small intestine. The objective of this work was to determine if peripheral blood B and T cells modify gene expression in response to a 6-week gluten challenge in patients with treated CeD, and to correlate any changes in peripheral B- or T-cell gene expression with the extent of gluten-induced histological damage to the small intestine.

Materials and Methods

All authors had access to the study data and reviewed and approved the final manuscript.

Abbreviations used in this paper: **Cd, crypt depth; CeD, celiac disease; cRNA, complementary RNA; TG2, transglutaminase 2; TR, T-cell receptor; Vh, villus height; Vh:Cd, ratio of villus height to crypt depth.**

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