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COMMENTARY

Insights into Environmental Factors Impacting Celiac Disease

Microbiota Modulation of Disease Pathogenesis

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The pathogenesis of celiac disease (CD) is secondary to immune damage in the gastrointestinal (GI) tract that occurs when patients with HLA-DQ2 or -DQ8 are exposed to the protein gluten in food products that contain wheat, rye, and barley. These specific HLA class II genes bind and present deamidated gluten peptides to helper T cells and are necessary but not sufficient for the development of CD. Recently, new diagnoses of CD have increased substantially leading to the question of whether new environmental factors are now modifying disease susceptibility.¹ Potential factors include the timing of exposure to foods containing gluten, breast versus formula feeding, and the composition of the intestinal microbiota; however, data supporting the role of any of these environmental factors in CD is limited. In this issue of *The American Journal of Pathology*, an article by Galipeau et al² utilizes a humanized mouse model of CD to strongly support a role for intestinal microbiota in contributing to gluten immune responses and CD-like pathology.²

The Intestinal Microbiome and Celiac Disease

There is now growing evidence that bacteria colonizing the GI tract, the intestinal microbiome, can influence both the development of the normal immune system and autoimmune diseases.³ One autoimmune disease that has been shown to have altered microbiota is type 1 diabetes (T1D), which often co-occurs with CD.^{4,5} In T1D, the non-obese diabetic (NOD) mouse model has recently been used to demonstrate that diet can impact both the incidence of disease and the composition of the microbiota.⁶ Mouse models and human patients with T1D when compared to control patients have lower populations of Firmicutes and

increased *Bacteroides*.⁶ A similar dysbiosis has been described in some but not all CD patients, and consequently, a typical CD microbiota profile has not been established. This leads some to question the role of intestinal microbiota in CD.⁷ This uncertainty highlights the difficulties encountered when studying the microbiota and its impact on human disease.

In human patients, there are multiple parameters that impact the variance in microbial populations. These include the extremely varied and inconsistent diet of human patients, differences in the location of the sample tested (duodenal biopsy versus feces), and multiple experimental approaches to microbial analysis (culture, PCR, 16sRNA sequencing).⁸ Consequently, the influence of microbiota on the incidence and pathogenesis of CD is still ambiguous.

Microbiota Manipulation in the NOD/DQ8 Model of CD

One approach to investigating the role of microbiota in disease is to use animal models that can be housed in a controlled environment. One of the most widely used mouse models of CD is the humanized mouse that expresses HLA-DQ8 on the non-obese diabetic (NOD) background (NOD/DQ8).⁹ This

A guest editor acted as the Editor-in-Chief for this manuscript. No person at University of Alabama at Birmingham was involved in the peer review process or final disposition.

Accepted for publication September 4, 2015.

Disclosures: None declared.

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model lacks all mouse endogenous class II molecules and develops barrier dysfunction and moderate enteropathy after gliadin-sensitization. This barrier dysfunction is also seen in CD patients, and it has been hypothesized that changes in intercellular tight junctions result in increased permeability to dietary gluten.¹⁰

Galipeau et al² investigated the role of microbiota in CD through a set of experiments that directly controlled and, subsequently, manipulated the microbiota in this mouse model.² First, germ-free (ie, bacteria free) NOD/DQ8 mice were generated. Compared to mice in their conventional facility, the animals raised in germ-free conditions manifested a strong celiac-like enteropathy in response to gluten (increased intra-epithelial lymphocytes, enterocyte apoptosis, and gliadin specific immune responses). These enhanced responses were not observed when gliadin was administered to mice conventionalized with clean specific pathogen free (SPF) flora, or altered schaedler flora, a mixture of eight individual bacterial strains primarily composed of *Lactobacillus*, *Bacteroides*, and *Clostridia*, with *Lactobacillus murinus* being the predominant organism in the upper GI tract.¹¹ A comparison of these clean mice with conventional SPF mice indicated that conventional SPF mice had more Proteobacteria, and additional experiments that manipulated the microbiota to expand Proteobacteria resulted in more severe gliadin-associated immune responses and enteropathy. The results from the controlled manipulation of the microbiota suggest that on a susceptible genetic background, the microbiome can be a disease instigating signal for CD.

Unanswered Questions

The findings of Galipeau et al² implicate opportunistic pathogens belonging to the Proteobacteria phylum in CD disease; however, they do not indicate that Proteobacteria cause CD. Instead, these findings reveal multiple avenues of investigation into the potential mechanisms by which Proteobacteria phylum organisms enhance the exposure and immune response to gliadin. Early models of intestinal dietary antigen exposure indicated that modulators of the intestinal immune response could trigger antigen-specific T cell responses and intestinal pathology in animals that were normally tolerant to oral antigens.¹² Therefore, one potential hypothesis is that organisms in the Proteobacteria phylum are actually impacting the development of CD by direct modulation of the immune response. Proteobacteria clearly increase inflammatory cytokines, which could enhance Th1 and Th17 responses by modulating antigen presentation and T cell function.¹³ However, the impact of the Proteobacteria may not be direct, instead they could modulate both the composition of the microbiota and the protective epithelial barrier. As an example, it has recently been described that mice with higher levels of Proteobacteria had a colonic mucus layer that was more penetrable to bacteria.¹⁴ This would clearly allow increased antigen access and therefore enhanced immune responses in the presence of Proteobacteria. However, as the

microbial sequence data was generated from cecal and fecal samples,² the composition of the microbiota in the small intestine and in the mucus barrier close to the small intestinal epithelial cell barrier is unknown. Its role in CD remains undiscovered.¹⁵

Although plausible mechanisms for the impact of the Proteobacteria phylum can be generated, the specific role of Proteobacteria should not be overinterpreted. A major caveat to the Proteobacteria hypothesis is that there is a vast difference between the eight species in altered schaedler flora and the natural microbiota seen in the conventional SPF mice, and in all likelihood, many taxa aside from the Proteobacteria phylum may contribute to the observed effects. One alternative hypothesis is generated from the analysis of the microbiota in conventional NOD/DQ8 feces before and after antibiotic treatment.² Mice treated with vancomycin had significantly higher levels of Proteobacteria (*Escherichia*, *Helicobacter*, and *Pasteurella*), but they also had dramatically lower levels of *Bacteroides* and *Parabacteroides*. As *Bacteroides* colonization has been shown to induce regulatory T cell differentiation in the intestine and oral administration of *Parabacteroides* can attenuate colitis and dampen the production of proinflammatory cytokines, it is clear that further studies need to include investigations of these (and other) bacteria to completely understand the role of microbiota in the modulation and treatment of CD.^{16,17}

Conclusions and Future Directions

The strength of this humanized mouse model is the ability to precisely control, manipulate, and analyze the intestinal microbiota. This strength can now be utilized to investigate mechanisms underlying the impact of microbiota on CD. Avenues of investigation should include the impact of specific microbial phyla on the epithelial barrier (including mucus composition and function, IgA levels, intraepithelial lymphocytes function, and antimicrobial peptide production), as well as the role that this dysbiotic microbiota plays in the development of the intestinal innate immune system, including innate lymphoid cells.¹⁸ This model will also allow for evaluation of potential therapeutic manipulations, such as the administration of probiotics that could induce regulatory T cells or fecal transplants to reshape the entire intestinal microbiota.^{19,20} However, newer technologies, such as the incorporation of data on the intestinal metabolome (microbial and host DNA sequences and metabolites) and the ability to harness new stem cell technology to develop human intestinal organoids for the study of complex intestinal diseases, should also be utilized to investigate the interrelationship between the microbiota and CD.^{21,22} In addition, the demonstration that microbiota impacts the development of CD now allows for further evaluation of human (and animal) antimicrobial immune responses to search for potential biomarkers to predict disease development.²³ Elucidating the exact mechanism(s) by

which the microbiota can modulate CD is of critical importance to future advances in diagnosis and treatment of CD.

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