"RESEARCH DIGEST": TRIMESTRAL BULLETIN ON BREAKTHROUGH PAPERS FOR CD

By the CD-SIG steering committee

TITLE / AUTHORS / JOURNAL	ABSTRACT
GLUTEN-FREE DIET INDUCES RAPID CHANGES IN PHENOTYPE AND SURVIVAL PROPERTIES OF GLUTEN-SPECIFIC T CELLS IN CELIAC DISEASE **Louise F Risnes , Henrik M Reims, Ronan M Doyle , Shuo-Wang Qiao, Ludvig M Sollid, Knut E A Lundin, Asbjørn Christophersen** **Gastroenterology. 2024 Mar 27:S0016-5085(24)00351-2.** **PMID: 38552723** **DOI: 10.1053/j.gastro.2024.03.027**	*Background and aims:* The treatment of celiac disease (CeD) with gluten-free diet (GFD) normalizes gut inflammation and disease-specific antibodies. CeD patients have HLA-restricted, gluten-specific T cells persisting in the blood and gut even after decades of GFD, which are re-activated and disease driving upon gluten exposure. Our aim was to examine the transition of activated gluten-specific T cells into a pool of persisting memory T cells concurrent with normalization of clinically relevant biomarkers during the first year of treatment. *Methods:* We followed 17 CeD patients during their initial GFD year, leading to disease remission. We assessed activation and frequency of gluten-specific CD4+ blood and gut T cells with HLA-DQ2.5:gluten tetramers and flow cytometry, disease-specific serology, histology and symptom scores. We assessed gluten-specific blood T cells within the first three weeks of GFD in six patients and serology in additional nine patients. *Results:* Gluten-specific CD4+ T cells peaked in blood at day 14 while upregulating Bcl-2 and downregulating Ki-67, then decreased in frequency within 10 weeks of GFD. CD38, ICOS, HLA-DR and Ki-67 decreased in gluten-specific cells within three days. PD-1, CD39 and OX40 expression persisted even after 12 months. IgA-TG2 decreased significantly within four

	weeks. *Conclusion:* GFD induces rapid changes in phenotype and number of gluten-specific CD4+ blood T cells, including a peak of non-proliferating, non-apoptotic cells at day 14. Subsequent alterations in T-cell phenotype associate with the quiescent but chronic nature of treated CeD. The rapid changes affecting gluten-specific T cells and disease-specific antibodies offer opportunities for clinical trials aiming at developing non-dietary treatments for newly diagnosed CeD patients.
PREDOMINANTLY ANTIBODY DEFICIENCY AND THE ASSOCIATION WITH CELIAC DISEASE IN SWEDEN: A NATIONWIDE CASE-CONTROL STUDY **Daniel V DiGiacomo, Bjorn Roelstraete, Benjamin Lebwohl, Peter H R Green, Lennart Hammarström, Jocelyn R Farmer, Hamed Khalili, Jonas F Ludvigsson** **Ann Allergy Asthma Immunol. 2024 Jan 23:S1081-1206(24)00027-9** **PMID: 38331244** **DOI:10.1016/j.anai.2024.01.019**	*Background:* Predominantly antibody deficiency (PAD) is associated with non-infectious inflammatory gastrointestinal (GI) disease. Population estimates of celiac disease (CeD) risk in those with PAD are limited. *Objective:* Estimate population risk of PAD in individuals with CeD. *Methods:* We conducted a nationwide case-control study of Swedish individuals who received a diagnosis of CeD between 1997 and 2017 (n=34,980), matched to population comparators by age, sex, calendar year, and county. CeD was confirmed through the Epidemiology Strengthened by histopathology Reports in Sweden (ESPRESSO) study, which provided information on biopsy specimens from each of Sweden's pathology departments. PAD was identified using International Classification of Diseases (ICD) 10th Revision coding and categorized according to the International Union of Immunologic Societies (IUIS). Logistic regression was used to calculate adjusted odds ratios (aORs) and 95% confidence intervals (CIs). *Results:* PAD was more prevalent in CeD as compared to population controls (n=105 (0.3%) vs n=57 (0.033%),

	respectively). This translated to an aOR of 8.23 (95%CI 5.95-11.48). The association was strongest with common variable immunodeficiency (CVID) (aOR 17.25; 95%CI 6.86-52.40), and slightly lower in other PAD (aOR 8.39; 95%CI 5.79-12.32). The risk of CeD remained increased ≥ 5 years after diagnosis of PAD (aOR 4.79; 95%CI 2.89-7.97, p-heterogeneity <0.001). *Conclusion:* PAD was associated with an increased risk of CeD. A particularly strong association was seen in those with CVID, although should be interpreted cautiously given the limited understanding of the mechanisms of histopathologic changes in these patients.
IL-10-PRODUCING REGULATORY CELLS IMPACT ON CELIAC DISEASE EVOLUTION **Laura Passerini, Giada Amodio, Virginia Bassi, Serena Vitale, Ilaria Mottola, Marina Di Stefano, Lorella Fanti, Paola Sgaramella, Chiara Ziparo, Silvia Furio, Renata Auricchio, Graziano Barera, Giovanni Di Nardo, Riccardo Troncone, Carmen Gianfrani, Silvia Gregori** **Clin Immunol. 2024 Feb 4:260:109923** **PMID: 38316201** **DOI: 10.1016/j.clim.2024.109923**	Celiac Disease (CD) is a T-cell mediated disorder caused by immune response to gluten, although the mechanisms underlying CD progression are still elusive. We analyzed immune cell composition, plasma cytokines, and gliadin-specific T-cell responses in patients with positive serology and normal intestinal mucosa (potential-CD) or villous atrophy (acute-CD), and after gluten-free diet (GFD). We found: an inflammatory signature and the presence of circulating gliadin-specific IFN-γ^+ T cells in CD patients regardless of mucosal damage; an increased frequency of IL-10-secreting dendritic cells (DC-10) in the gut and of circulating gliadin-specific IL-10-secreting T cells in potential-CD; IL-10 inhibition increased IFN-γ secretion by gliadin-specific intestinal T cells from acute- and potential-CD. On GFD, inflammatory cytokines normalized, while IL-10-producing T cells accumulated in the gut. We show that IL-10-producing cells are fundamental in controlling pathological T-cell responses to gluten: DC-10 protect the intestinal mucosa from damage and represent a marker of potential-CD.

GENOTYPES PREDISPOSING FOR CELIAC DISEASE AND AUTOIMMUNE DIABETES AND RISK OF INFECTIONS IN EARLY CHILDHOOD

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Objectives: Infections in early childhood have been associated with risk of celiac disease (CD) and type 1 diabetes (T1D). We investigated whether this is driven by susceptibility genes for autoimmune disease by comparing infection frequency by genetic susceptibility variants for CD or T1D.

Methods: We genotyped 373 controls and 384 children who developed CD or T1D in the population-based Norwegian Mother, Father and Child Cohort study (MoBa) study for human leukocyte antigen (HLA)-DQ, FUT2, SH2B3, and PTPN22, and calculated a weighted non-HLA genetic risk score (GRS) for CD and T1D based on over 40 SNPs. Parents reported infections in questionnaires when children were 6 and 18 months old. We used negative binomial regression to estimate incidence rate ratio (IRR) for infections by genotype.

Results: HLA genotypes for CD and T1D or non-HLA GRS for T1D were not associated with infections. The non-HLA GRS for CD was associated with a nonsignificantly lower frequency of infections (aIRR: 0.95, 95% CI: 0.87-1.03 per weighted allele score), and significantly so when restricting to healthy controls (aIRR: 0.89, 0.81-0.99). Participants homozygous for rs601338(A;A) at FUT2, often referred to as nonsecretors, had a nonsignificantly lower risk of infections (aIRR: 0.91, 95% CI: 0.83-1.01). SH2B3 and PTPN22 genotypes were not associated with infections. The association between infections and risk of CD (OR: 1.15 per five infections) was strengthened after adjustment for HLA genotype and non-HLA GRS (OR: 1.24).

Conclusions: HLA variants and non-HLA GRS conferring susceptibility for CD were not associated with increased risk of infections in early childhood and is unlikely to drive the

	observed association between infections and risk of CD or T1D in many studies.
BIOPSY PROTEOME SCORING TO DETERMINE MUCOSAL REMODELING IN CELIAC DISEASE. **Anette Johansen, Geir Kjetil F Sandve, Jostein Holen Ibsen, Knut E A Lundin, Ludvig M Sollid, Jorunn Stamnaes** **Gastroenterology 2024 Mar 11:S0016-5085(24)00286-5.** **PMID: 38467384** **DOI: 10.1053/j.gastro.2024.03.006**	*Background & aims*: Histologic evaluation of gut biopsy specimens is a cornerstone for diagnosis and management of celiac disease (CeD). Despite its wide use, the method depends on proper biopsy orientation, and it suffers from interobserver variability. Biopsy proteome measurement reporting on the tissue state can be obtained by mass spectrometry analysis of formalin-fixed paraffin-embedded tissue. Here we aimed to transform biopsy proteome data into numerical scores that give observer-independent measures of mucosal remodeling in CeD. *Methods:* A pipeline using glass-mounted formalin-fixed paraffin-embedded sections for mass spectrometry-based proteome analysis was established. Proteome data were converted to numerical scores using 2 complementary approaches: a rank-based enrichment score and a score based on machine-learning using logistic regression. The 2 scoring approaches were compared with each other and with histology analyzing 18 patients with CeD with biopsy specimens collected before and after treatment with a gluten-free diet as well as biopsy specimens from patients with CeD with varying degree of remission (n = 22). Biopsy specimens from individuals without CeD (n = 32) were also analyzed. *Results*: The method yielded reliable proteome scoring of both unstained and H&E-stained glass-mounted sections. The scores of the 2 approaches were highly correlated, reflecting that both approaches pick up proteome changes in the same biological pathways. The proteome scores correlated with villus height-to-

	crypt depth ratio. Thus, the method is able to score biopsy specimens with poor orientation. *Conclusions:* Biopsy proteome scores give reliable observer and orientation-independent measures of mucosal remodeling in CeD. The proteomic method can readily be implemented by nonexpert laboratories in parallel to histology assessment and easily scaled for clinical trial settings.
ZONULIN AS A BIOMARKER FOR THE DEVELOPMENT OF CELIAC DISEASE **Tracey M DaFonte, Francesco Valitutti, Victoria Kenyon, Joseph J Locascio, Monica Montuori, Ruggiero Francavilla, Tiziana Passaro, Marco Crocco, Lorenzo Norsa, Pasqua Piemontese, Mariella Baldassarre, Alessio Fasano, Maureen M Leonard; CD-GEMM Study Group** **Pediatrics 2024 Jan 1;153(1):e2023063050.** **PMID:** 38062791 **DOI: 10.1542/peds.2023-063050**	*Objectives:* Increased intestinal permeability seems to be a key factor in the pathogenesis of autoimmune diseases, including celiac disease (CeD). However, it is unknown whether increased permeability precedes CeD onset. This study's objective was to determine whether intestinal permeability is altered before celiac disease autoimmunity (CDA) in at-risk children. We also examined whether environmental factors impacted zonulin, a widely used marker of gut permeability. *Methods:* We evaluated 102 children in the CDGEMM study from 2014-2022. We included 51 CDA cases and matched controls, who were enrolled for 12 months or more and consumed gluten. We measured serum zonulin from age 12 months to time of CDA onset, and the corresponding time point in controls, and examined clinical factors of interest. We ran a mixed-effects longitudinal model with dependent variable zonulin. *Results*: Children who developed CDA had a significant increase in zonulin in the 18.3 months (range 6-78) preceding CDA compared to those without CDA (slope differential = β = 0.1277, 95% CI: 0.001, 0.255). Among metadata considered, zonulin

	trajectory was only influenced by increasing number of antibiotic courses, which increased the slope of trajectory of zonulin over time in CDA subjects (P = .04). *Conclusions:* Zonulin levels significantly rise in the months that precede CDA diagnosis. Exposure to a greater number of antibiotic courses was associated with an increase in zonulin levels in CDA subjects. This suggests zonulin may be used as a biomarker for preclinical CeD screening in at-risk children, and multiple antibiotic courses may increase their risk of CDA by increasing zonulin levels.
NOVEL BACTEROIDES VULGATUS STRAIN PROTECTS AGAINST GLUTEN-INDUCED BREAK OF HUMAN CELIAC GUT EPITHELIAL HOMEOSTASIS: A PRE-CLINICAL PROOF-OF-CONCEPT STUDY **Tina Tran, Stefania Senger, Mariella Baldassarre, Rachel A Brosnan, Fernanda Cristofori, Marco Crocco, Stefania De Santis, Luca Elli, Christina S Faherty, Ruggero Francavilla, Isabella Goodchild-Michelman, Victoria A Kenyon, Maureen M Leonard, Rosiane S Lima, Federica Malerba, Monica Montuori, Annalisa Morelli, Lorenzo Norsa, Tiziana Passaro, Pasqua Piemontese James C Reed, Naire Sansotta, Francesco Valitutti, Ali R Zomorrodi, Alessio Fasano; CDGEMM Team** **Pediatr Res 2024 Jan 4.** **PMID:** 38177249 **DOI: 10.1038/s41390-023-02960-0**	*Background and aims:* We have identified a decreased abundance of microbial species known to have a potential anti-inflammatory, protective effect in subjects that developed Celiac Disease (CeD) compared to those who did not. We aim to confirm the potential protective role of one of these species, namely Bacteroides vulgatus, and to mechanistically establish the effect of bacterial bioproducts on gluten-dependent changes on human gut epithelial functions. *Methods:* We identified, isolated, cultivated, and sequenced a unique novel strain (20220303-A2) of B. vulgatus found only in control subjects. Using a human gut organoid system developed from pre-celiac patients, we monitored epithelial phenotype and innate immune cytokines at baseline, after exposure to gliadin, or gliadin plus B. vulgatus cell free supernatant (CFS). *Results:* Following gliadin exposure, we observed increases in epithelial cell death, epithelial monolayer permeability, and secretion of pro-inflammatory cytokines. These effects were

	mitigated upon exposure to B. vulgatus 20220303-A2 CFS, which had matched phenotype gene product mutations. These protective effects were mediated by epigenetic reprogramming of the organoids treated with B. vulgatus CFS. *Conclusions:* We identified a unique strain of B. vulgatus that may exert a beneficial role by protecting CeD epithelium against a gluten-induced break of epithelial tolerance through miRNA reprogramming. *Impact:* Gut dysbiosis precedes the onset of celiac disease in genetically at-risk infants. This dysbiosis is characterized by the loss of protective bacterial strains in those children who will go on to develop celiac disease. The paper reports the mechanism by which one of these protective strains, B. vulgatus, ameliorates the gluten-induced break of gut epithelial homeostasis by epigenetically re-programming the target intestinal epithelium involving pathways controlling permeability, immune response, and cell turnover.