THE ROLE OF EARLY VIRAL INFECTIONS AS A TRIGGER FOR CELIAC DISEASE DEVELOPMENT: A PROSPECTIVE STUDY IN THE EUROPEAN PREVENTCD PEDIATRIC COHORT

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Background

CD is a gluten-dependent inflammatory disorder currently affecting 1-2% of the general population[1]. Because of its raising prevalence, the social and psychological challenges related to the life-long adherence to a gluten free diet (GFD) and the increased risk of developing other associated autoimmune disorders[2], CD represents a major global health burden. The loss of oral tolerance (LOT) to dietary gluten is a key pathogenic event in CD, leading to an inflammatory anti-gluten T cell response and the production of anti-tissue transglutaminase 2 (TG2) antibodies. The presence of the HLA-DQ2 and/or DQ-8 alleles is required, although not sufficient, to mount such response[3]. Indeed, over 40% of individuals of Caucasian ancestry carry either one of these alleles, yet only 1-2% develops CD, suggesting that other factors contribute to trigger CD in susceptible individuals. Genetic, epidemiological and immunological studies support a role for viral infections in CD onset[4-7]. Work by the group of Bana Jabri recently showed that Reovirus[8] and Norovirus[9] infections can promote LOT in vivo. In particular, Reovirus T1L infection induced LOT to gluten in mice expressing the human HLA-DQ8, by promoting an intestinal inflammatory Th1 response via IRF1 and depressing regulatory FOXP3+ T cells via type-1 IFN[9], providing for the first time a mechanism behind viral-induced LOT. Whether other viral strains (and which) promote a LOT phenotype and CD development through similar or alternative immune pathways, remains to be determined. Importantly, over 20% of children at high genetic risk develop CD in the first three years of life[10], suggesting that LOT to gluten might occur in this time-window and preventive strategies should be precocious in this population. Early feeding strategies[11] modulating the amount and timing of first

gluten introduction failed to prevent CD onset in high-risk infants[10]. Nevertheless, the role of early viral infections in triggering LOT to gluten in a time-sensitive manner and with respect to first dietary gluten introduction, has not been investigated in high-risk infants. Hypothesis and Significance. Our overarching hypothesis is that viral infections leading to an alteration in the intestinal immune homeostasis around the time of weaning trigger LOT to dietary gluten and promote CD development in genetically susceptible individuals. Taking advantage of the data and biobank of the prospective PreventCD cohort10 we will define the prevalence, timing and association with gluten intake of early viral infections and their relation with LOT to gluten and CD development in high-risk children. Furthermore, we will investigate the nature and magnitude of the anti-viral immune response to reveal host-viral interactions that have a pathogenic role in promoting CD. Overall, we expect this study will help defining new strategies to prevent CD in high-risk subjects, including change of early feeding practices and vaccinations.

Study design

Aim 1. To outline the early viral contribution to LOT to gluten and CD onset among high-risk infants

1.1 To identify prospectively which viral infections in the first years of life are associated with LOT to gluten and CD development among high-risk infants.

1.2 To define an early time-window, with relation to first dietary gluten introduction and breastfeeding, during which viral infections are more likely to promote later CD development in high-risk infants.

Aim 2. To characterize the host-viral interactions that have a pathogenic association with CD onset.

2.1 To define the nature and magnitude of the peripheral anti-viral immune response associated with CD. 2.2 To determine the intestinal innate immune signature promoted by viral infections in the context of CD.

Study design. To test our hypothesis, we will take advantage of the biological samples collected in the context of the European PreventCD Study (www.preventcd.com)[10]. PreventCD (Partner 2) is an on-going multinational project initiated in 2007 under the auspices of the European Commission 6th FP program (FP6-2005-FOOD-4B-36383) whose purpose is to investigate the influence of genetic, immunological and environmental factors, with a focus on infant nutrition, on the development of CD and related autoimmune phenomena. This cohort includes over nine-hundred European infants enrolled in the different participating countries, carrying the HLA-

DQ2 and/or DQ8 alleles, who are first degree relatives of CD patients, thus being at increased risk of developing themselves CD. They were longitudinally followed-up since birth (current follow-up: 10 years) with clinical and laboratory assessments at 4, 6, 9, 12, 18, 24, 36, 48 months and annually thereafter. At the time of first anti-TG2 antibodies appearance, small intestinal biopsies were obtained to check for CD development. The availability of serial serum and blood samples collected at different time points before and after the diagnosis of CD, offers a unique opportunity to test the viral hypothesis. 80 PreventCD children who developed CD and an equal number of age and sex-matched PreventCD infants who did not develop CD, will be included in the study.

A comprehensive serological viral profiling of these infants (Aim 1.1) will be performed using a microarray technology implemented by one of our collaborators (M. Mina, partner 4) called VirScan[12-13]. This will allow us to identify viral candidates associated with LOT to gluten. To establish whether an early window of increased susceptibility to viral-promoted CD could be identified in a high-risk population, we will correlate the timing of occurrence of viral infections to dietary gluten introduction and development of LOT to gluten and/or villous atrophy (Aim 1.2).

Clinical and laboratory data will be unblinded only once Aim 1.1 will be finalized to guarantee rigor. As shown for Reovirus (Fig. 3), not only the exposure, but also the nature of the host-viral interaction by specific viral strains will influence disease development. Thus, we will investigate the quality of the host-viral interaction (Aim 2.1) by assessing the magnitude (antibody breadth and titres) and nature (Ig sub-classes) of the antibody response against specific viruses, and characterizing the specific epitopes for these antibodies, using recently implemented features of the VirScan (partner 3). The computation analysis will be performed by partner 3 (L. Barreiro at the UChicago) and partner 4 (M. Mina at Harvard) taking advantage of the pipelines already established in previously published work[12-13].

Moreover, we will study the CD-associate viral-induced gene transcriptional profile of PBMCs (Aim 2.1) using a combined approach: (1) bulk and single-cell RNA-seq of PBMCs (collaboration with partner 5, S. Withoff) and (2) RNA-seq upon purification of cell subsets. Finally, we will assess by immunohistochemistry and RNA-scope the expression in the duodenal biopsies of innate inflammatory molecules associated to viral exposure including IRF[18] (Fig. 1) MxA (Fig. 2) and other interferon sensitive genes, and will define their association with pathogenic viral exposures and CD development (Aim 2.2).

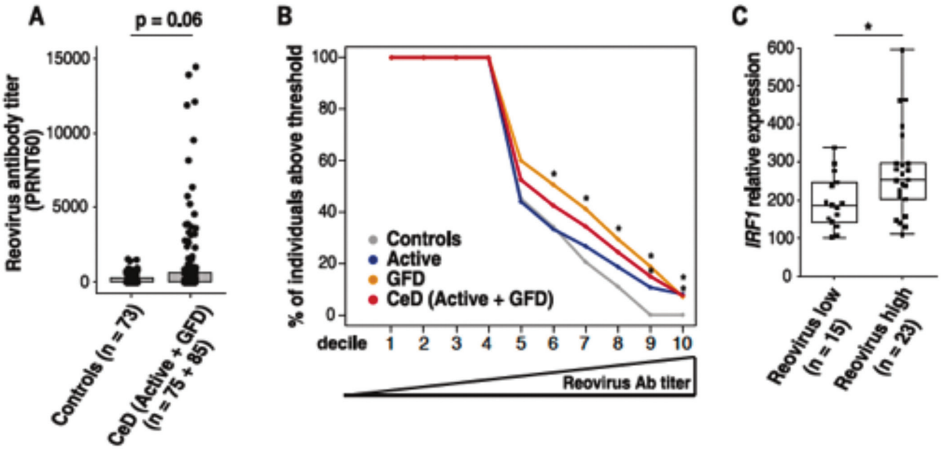

Figure 1. High Reovirus antibody titers in CD patients are associated with increased IRF1 duodenal expression. (A) Reovirus antibody (Ab) titers in control (n=73) and CeD patients (n=160). (B) Percentage of controls (grey), active CeD (blue), CeD patients on a gluten-free diet (GFD) (orange), and active + GFD combined (red) that have reovirus Ab titers above an increasingly higher cut-off (left to right), determined by the deciles of antibody titers distribution in the analyzed samples. GFD and all CeD patients (Active+GFD) are significantly overrepresented among those with titers above the 6th (PRNT60=156) and the 9th decile (PRNT60=1597), respectively. (C) IRF1 relative expression in small intestinal biopsies of GFD patients (n=38) with low or high (< or > PRNT60=47) reovirus antibody titers as analyzed by RT-PCR.

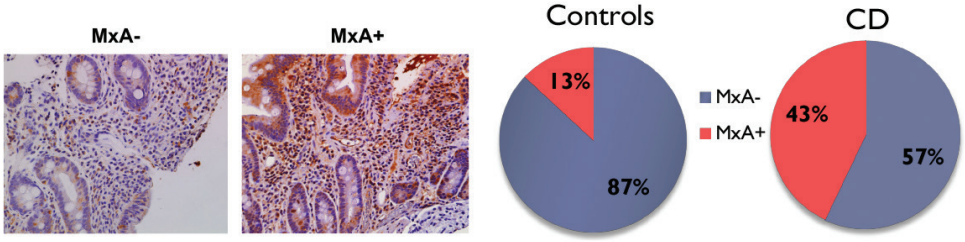

Figure 2. MxA is overexpressed in the gut of active CeD patients. Representative MxA immunohistochemical staining from duodenal biopsies from a control (left) and an active CeD patient (right). Quantification of the rate of MxA positive cells infiltrating the lamina propria, performed by counting the number of stained cells within the inflammatory infiltrate.

Future perspectives

Using VirScan we will comprehensively screen for early viral infections associated with CD in infants at high-risk and we expect to (I) identify new viruses that could be

found to be associated with Cd development, and thus possibly having a pathogenic role in high risk populations. (II) Define the window around gluten introduction at which viruses have a pathogenic potential, (III) confirm the association between previously reported infections and CD onset (i.e. Reovirus, Rotavirus), (IV) delineate the nature of the humoral immune response that is associated with LOT to gluten, by looking at the breadth, magnitude and the isotype of the antibody response. The availability of longitudinal samples will enable us to establish for each infant an individual serological profile, minimizing the noise deriving from interindividual variability and most importantly allowing to link the timing of the viral infection to LOT to gluten. Since CD-specific antibody titres (TG2) were assessed at each time point, we will define a sensitive time-window for early infections to promote LOT to gluten and CD onset. We will perform a transcriptome analysis of purified cells to maximize the data obtained while minimizing the biological samples used. In the intestinal biopsies we will detect a broad range of innate markers, associated to viral infections protein by IHC and mRNA by RNA-scope). Characterizing the peripheral and intestinal immune signature in response to CD-associated viral infections will help (1) confirm the pathogenic nature of the host-viral interactions linked to CD and (2) provide insights into the mechanisms underlying LOT to gluten.

Understanding the interaction between early viral infections, gluten introduction and CD onset will foster design of new preventive strategies, such as avoiding gluten introduction at times in which protective maternal antibodies are waning and/or designing vaccination strategies that could protect at-risk infants from developing CD upon specific infections. We believe our findings could be relevant for other autoimmune disorders that may be triggered by viral infections.

Publications

1. *Singh P, et al. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2018; 16(6):823-836.e2.*
2. *Troncone R and Discepolo V. Celiac Disease and Autoimmunity. J Pediatr Gastroenterol Nutr. 2014; 59:S9.*
3. *Abadie V et al. Integration of Genetic and Immunological Insights into a Model of Celiac Disease Pathogenesis. Annu. Rev. Immunol. 2011; 29:493-525.*
4. *CR Kahrs et al. Enterovirus as trigger of coeliac disease: nested case-control study within prospective birth cohort. BMJ 2019; 364:231.*
5. *Plot L and Amital H. Infectious associations of Celiac disease. Autoimmun Rev. 2009; 8, 316-319.*
6. *Smits, S. L. et al. Human astrovirus infection in a patient with new-onset celiac disease. J Clin Microbiol. 2010; 48, 3416-3418.*
7. *Stene LC et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 2006; 101, 2333-2340.*
8. *Bouziat R et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science. 2017; 356, 44-50.*

9. *Bouziat R et al. Murine Norovirus Infection Induces TH1 Inflammatory Responses to Dietary Antigens. Cell Host Microbe. 2018 14; 24(5):677-688.*

10. *Vriezinga SL et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med 371, 1304-1315, (2014).*

11. *Meijer CR et al. Does Infant Feeding Modulate the Manifestation of Celiac Disease and Type 1 Diabetes? Curr Opin Clin Nutr Metab Care. 2017; 20(3):222-226.*

12. *Xu GJ et al. Comprehensive serological profiling of human populations using a synthetic human virome. Science. 2015 5; 348(6239).*

13. *Harrison GF et al. Natural selection contributed to immunological differences between hunter-gatherers and agriculturalists. Nat Ecol Evol. 2019; 3(8):1253-1264.*

External collaborations

- *Leading Center: ELFID. Co-Leading Center: Department of Medicine, University of Chicago*
- *Partner 1. Bana Jabri. Director of Research at the Celiac Disease Center and Vice Chair of Medicine, University of Chicago*
- *Partner 2. Prevent CD Group. (Coordinated by Luisa Mearin, Leiden University Medical Center). The PreventCD project involves 17 members, including among others: Italy, Sweden, Poland, Spain, Germany, Norway, Croatia, Hungary, Belgium, Israel and is coordinated in the Netherlands. Biological samples collected in the context of this study will be sent to the Naples (Leading Center) and Chicago (Partner 1) for processing. The PreventCD group will also provide patients' clinical and laboratory data*
- *Partner 3. Michela Mina's lab (Harvard University).*
- *Partner 4. Luis Barreiro's lab (UChicago)*
- *Partner 5. Sebo Withoff's lab (Groningen)*