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HUMANIZED CELIAC-PRONE EPITHELIUM *IN VITRO* EXPRESS MHC-II AND CO-STIMULATORY MOLECULES NECESSARY FOR GLUTEN PEPTIDE PRESENTATION

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Background: The role intestinal epithelial cells (IECs) play in the breakdown of tolerance to gluten at an early stage in celiac disease (CeD) is unclear. Epithelial stress is a feature of CeD, and although the triggers are largely unknown, it is accompanied by expression of several markers that could be involved in initiation of inflammatory responses. IECs have been shown to express MHC class II (MHC-II) molecules and participate in antigen presentation in several models. Whether IECs can participate in gluten peptide presentation, the major environmental trigger in celiac disease, is unknown. To study this, a model expressing human MHC-II, HLA DQ8 or HLA-DQ2, would be required.
Aims: To develop organoid monolayers from transgenic mice expressing human celiac risk genes: HLA-DQ8 and -DQ2. To investigate conditions leading to the induction of epithelial MHC-II and its main co-stimulatory molecules, CD80, CD86 and CD40, that could enable early gluten peptide presentation.
Methods: In order to show pathophysiological significance of the model, we used two approaches, either induction of inflammation *in vivo* through gluten sensitization, or direct stimulation of the monolayers using pro-inflammatory cytokines relevant in CeD, such as IFNγ. Mice were sensitized with Pepsin-Trypsin digested gliadin and cholera toxin (CT) once a week for 3 weeks, followed by a challenge phase in which they only received gliadin. Control mice received CT only. We then developed organoid monolayers from the duodenum followed by stimulation with 10 ng/ml IFNγ. Finally, markers necessary for gluten peptide presentation, the expression of MHC-II and its co-stimulatory molecules, were evaluated using flow cytometry.
Results: Both *in vivo* gluten sensitization and *in vitro* stimulation of the organoid derived monolayer with IFNγ induced a proinflammatory response, that independently

primed the epithelium to express MHC-II molecules ($p = 0.02$ and < 0.0001, respectively). When *in vivo* sensitization and *in vitro* IFNγ stimulation were combined, epithelial MHC-II expression was further upregulated ($p < 0.0001$). Lastly, only the combination of gluten sensitization and *in vitro* IFNγ induced expression of MHC-II co-stimulatory molecules, which are necessary for antigen presentation.
Conclusions: Our findings support that gluten induced-inflammation *in vivo* as well as independent stimuli that release IFNγ enhance the capacity of the IECs to express MHC-II molecules. However, co-stimulatory molecules are only expressed by the epithelium when both gluten tolerance is broken by *in vivo* sensitization and the organoid monolayers is further exposed to IFNγ. The results support the hypothesis that the epithelium participates in gluten peptide presentation and that this pathway is stimulated by both gluten-dependent and independent inflammation.

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