COELIAC DISEASE (MECHANISMS)

INTERACTION OF FOOD AND INTESTINAL EPITHELIUM IN PHYSIOLOGY AND DISEASE

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Background

Inflammatory response represents the "common soil" of many multifactorial diseases, that have increased incidence, including type 2 diabetes, cardiovascular and neurodegenerative diseases, inflammatory bowel diseases, obesity, cancer, asthma, and ageing. A wide set of nutrients such as Carbohydrates, Lipids and even Salt have been shown to induce inflammation in several tissues and more in general western diet and life stile have been linked to cellular inflammation. Intestinal epithelium is the first tissue to be hit by inflammatory nutrients in several diseases including diabetes.

Celiac Disease (CD) is an ideal model of disease induced by food. CD is an autoimmune disease characterized by an enteropathy with inflammatory and structural changes causing the remodelling of the small intestinal mucosa. These changes are due to the loss of oral tolerance to gluten, a protein contained in wheat, barley and rye. The mucosal inflammation results from a Th1 response to gliadin peptides (e.g., the 33-mer A-gliadin peptide) presented by HLA-DQ2 or 8 (human leukocyte antigen) and activation of innate immune pathways. Several factors contribute to this activation, including other gliadin peptides, e.g., A-gliadin peptide P31-43, not presented by HLA-DQ2 or 8. Both the 33-mer and 25-mer (P31-55), containing the peptides

P57-68 and P31-43 respectively, are not efficiently hydrolysed by gastric, pancreatic and intestinal proteases. Thus, these peptides are active in vivo in the intestine after gluten ingestion. Our working hypothesis was that gliadin can induce cellular inflammation and that in CD there is a constitutive inflammation.

Main achievements

1) Gliadin peptides can induce cellular inflammation and activation of innate immunity

P31-43 induces the innate immune response and enterocyte proliferation delaying the endocytic trafficking. In both celiac enterocytes and CaCo-2 cells, P31-43 localizes to the early endosomes. P31-43 shares sequence similarity to HRS (growth factor-regulated tyrosine kinase substrate) a key molecule involved in regulating endocytic maturation and located on the membranes of early endocytic vesicles. P31-43, in CaCo-2 cells, interferes with the correct localization of HRS in early endosomes. Therefore P31-43 induces two important effects, on one side it delays endocytic maturation and on the other side it alters the recycling pathway. A delay in endocytic maturation can reduce the degradation of epidermal growth factor receptor (EGFR), which is endocytosed in these vesicles. This delay prolongs its activation, with consequent increased proliferation, actin remodelling and other biological effects. On the other side alterations in the recycling pathway direct more IL15R-α (IL15-receptor-α) to the cell surface, increasing trans presentation, in the epithelial cells of IL15/IL15R-α. In CD, type 1 interferons also play a role in the loss of oral tolerance to gluten. In fact rotavirus infections are associated with an increased incidence of CD; IFN-α therapy can induce CD in some genetically susceptible individuals and IFN-α (interferon-α) expression is dysregulated in patients with CD. Moreover gliadin peptide, P31-43 activated IFN-α, a mediator of the innate immune response in CD, in the intestine of subjects with CD and an enterocyte cell line, CaCo-2. P31-43 cooperated with a viral ligand to activate the TLR7 pathway by interfering with endocytic trafficking. Based on these results, the vesicular pathway regulates the innate/inflammatory response to viral ligands and bioactive dietary peptides, suggesting that together with viral infections, alimentary proteins able to mimic and potentiate the innate immune response to viruses, can trigger an autoimmune disease such as CD.

2) In CD cells there is a constitutive inflammation and activation of the innate immunity

A stressed/inflamed celiac cellular phenotype in enterocytes and fibroblasts probably due to an alteration in the early-recycling endosomal system has been recently described. Celiac cells are more sensitive to the gliadin peptide P31-43 and IL15 than controls. This phenotype is reproduced in control cells by inducing a delay in early

vesicular trafficking. This constitutive lesion might mediate the stress/innate immune response to gliadin, which can be one of the triggers of the gliadin-specific T-cell response.

Future perspectives

1) CD cellular phenotype in the natural history of CD

Constitutive alterations have been described both in cells and in biopsies from CD patients, that can be grouped in three sets: alterations of the cell structure (cell shape, actin modifications, increased permeability and vesicular trafficking alterations), of signaling/proliferation pathways, and of stress/innate immunity activation. These constitutive alterations, that described a "CD cellular phenotype", can be found in CD patients in remission phase, and in CD cells, like the skin fibroblasts, that are far from the primary site of inflammation and in conditions of absence of gluten. Our hypothesis is that these alterations are constitutive and already present in the very early phases of the disease. To test this hypothesis we will study biopsies from CD patients, not only in the acute, the remission phase of the disease, also before the evolution to villous atrophy in potentials CD patients for: a) structural alterations of the epithelium, in particular, intestinal epithelium integrity and vesicular trafficking. b) Signaling and proliferation. c) stress/innate immunity activation. Moreover we will extend these observations to the first degree relatives of CD patients and in children at risk for the disease to confirm the hypothesis that these alterations are constitutive and have a role in the pathogenesis of the disease.

2) Cellular models of intestinal epithelium

To study the interaction between food and intestinal epithelium we have set up several cellular models that can be challenged and than observed for different read outs. Among these models we have developed intestinal "organoides" from intestinal biopsies both from humans and mice. Intestinal organoides resemble the intestinal epithelium with crypts and villi, globet, enterochromaffin and Paneth's cells. They allow to study cell morphology, signalling, intestinal permeability, inflammation and intestinal integrity in the epithelium of normal and diseased subjects. Thanks to our intestinal epithelium models we will be able to study different diseases related to inflammation and food including diabetes and celiac disease. We have already isolated organoides from CD patients at different stage of the disease (Active, Remission, Potentials) and studied the their use as an intestinal model for CD, reproducing the main features of the CD intestinal mucosa including alterations of the vesicular trafficking, proliferation, activation of the innate immunity and inflammation. Moreover organoides can be a good model to study the role of non-HLA genes in the intestinal lesion typical of CD mucosa.

3) In vitro model of digestion

We have developed in collaboration with Prof Nigro from DICMAPI of the University of Naples Federico II, an in vitro dynamic digestion system. This system, was able to digest simple starches and a more complex food in the correct compartments. In the future we would like to use this system to understand the effect of digestion on food before interacting with the intestinal epithelium.

4) Longevity pathway

Our intestinal models, including organoides form normal intestine and disease and in vitro model of digestion, are good candidates to test the interaction between food and intestinal epithelium. In particular we will test food, functional food and nutraceuticals able to mimic caloric restriction (CRM). CR has a variety of effects on extending lifespan and delaying the onset of age-related diseases, and it is accepted as the only established experimental antiaging intervention. The nutrient-sensing pathways including those involving sirtuins (especially SIRT1) and mammalian target of rapamycin (mTOR) may regulate the physiology of CR, and candidate CRMs that modulate these specific pathways have been identified and investigated using cellular and animal models.

Publications

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