PROBIOTICS AND GLIADIN PEPTIDES BIOLOGICAL ACTIVITY

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Background

Celiac Disease (CD) is both a frequent disease (1:100) and an interesting model of a disease induced by food. Undigested gliadin peptides are able to stimulate both innate and adaptive immune response and to cause damage to the intestinal mucosa of CD patients. GFD exposes celiac patients to nutritional deficiency. In fact, the consumption of some nutrients, particularly fibers, iron, calcium and folate, is lower than normal in patients who adhere to GFD. Alternative therapies for CD have been proposed, in particular one of this is focused on the destruction of gliadin peptides present in the food, while another approach has the goal of blocking the entry of peptides in the intestinal epithelium, preventing the activation of the immune response. Probiotics have characteristics that could be useful in both these areas.

Activation of innate immunity by gliadin peptides is an important component of the early events of the disease. In particular, the ''toxic'' A-gliadin peptide P31-43 induces several pleiotropic effects including Epidermal Growth Factor Receptor (EGFR)-dependent actin remodelling and proliferation in cultured cell lines and in enterocytes from CD patients. Moreover, P31-43 can induce a delay of the endocytic trafficking probably due to a sequence similarity to HRS, a nodal protein that regulates the maturation from early to late vesicles.

Microbial dysbiosis has been largely reported in CD patients. A peculiar Neisseria flavescens (Nf) strain in adult CD patients (CD-Nf) has been previously identified This bacterial strain, isolated from the above samples, induced an immune-inflammatory response in human and murine dendritic cells, in CaCo-2 cells and in ex-vivo duodenal mucosal explants of control subjects (Ctr), suggesting that it could play a role in CD.

Main achievements

1) Lactobacillus paracasei CBA L74 interferes with gliadin peptides entrance in Caco-2 cells

We studied the effect of probiotic LP CBA L74 on the entrance of both P31-43 and P57-68, the two main undigested peptides involved in CD pathogenesis, in Caco2 cells. We showed that both LP CBA L74 at different concentrations and its supernatant are able to reduce the peptides entrance in Caco-2 cells. Filtered supernatant of probiotics has the same biological effect of wild complete probiotic: this evidence suggests that the component responsible of biological effect is secreted in the supernatant. We have also evaluated the ability of the probiotic to ferment cereals. Oats, rice and wheat were fermented with LP CBA L74 efficiently, and the fermented cereals were able to interfere with P31-43-liss entrance. Fermentation process is thought to render cereals more digestible; our data add a new property to LP CBA L74-fermented cereals: the interference with gliadin peptides entrance. Moreover, the fermented cereals have more chance to be active at the level of the intestinal mucosa with respect to the probiotic that very seldom can colonize the intestine. Therefore, probiotic LP CBA L74-fermented food has a potential role to prevent the toxic effects of undigested gliadin peptides.

2) Celiac disease associated Neisseria flavescens decreases mitochondrial respiration in CaCo 2epithelial cells. Impact of Lactobacillus paracasei CBA L 74 on bacterial induced cellular imbalance

P31 43 toxic gliadin peptide could influence the intracellula r trafficking of CD N. flavescens and Ctr N. flavescens. The treatment of N. flavescens infected CaCo 2 cells with the P31 43 peptide increased the colocalization of N. flavescens strains with the EEA1 marker, which confirms that P31 43 can delay vesicular trafficking irrespective of the vesicle cargo. It is conceivable that N. flavescens strains and the P31-43 peptide cooperate to delay endocytic trafficking at the level of the early compartment thereby providing a “comfort zone” to enable bacteria to survive longer in the cells. Notably, P31-43 increased the co-localization of the N. flavescens isolates with LC3-positive vesicles, but not with LAMP-2-positive compartments, which suggests that also LC3 vesicles cannot reach the late degradative vesicles. Although L. paracasei CBA probiotic supernatant did not alter entry of the bacteria into the cell, it reduced the bacterium viability.
Furthermore, L. paracasei CBA probiotic supernatant significantly reduced and increased the co localization of CD N. flavescens with EEA1 and with LAMP 2 markers, respectively, despite the presence of the P31 43 peptide. The Neisseria flavescens strain induces imbalance in the mitochondrial respiration of CaCo-2 epithelial cells in parallel to the inflammation-immune response. This metabolic alteration appears

to be in part reversed by the probiotic L. paracasei-CBA, irrespective of the presence of the P31-43 peptide.

Future perspectives

Cells and organisms need to integrate information from the environment to ensure that they only grow when conditions are favorable. The highly conserved Ser/Thr protein kinase target of rapamycin (TOR) is a key integrator of environmental cues, including nutrient and growth factor availability as well as stress. Under nutrient-rich conditions, TOR promotes cell growth by stimulating biosynthetic pathways, including protein synthesis, and by inhibiting cellular catabolism such as through repression of the autophagy pathway. The opposite happens in conditions or caloric restriction.

1) What is the element present in the Lactobacillus Paracasei supernatant that is able to prevent gliadin effects on CaCo2 cells? We will start from the hypothesis that the active element in the LB supernatants is of proteic origin. We have previously shown that lactobacillus paracasei effects on gliadin peptide entrance in CaCo2 cells was probably mediated by a protein. For this reason, we will test on Caco2 cells the effect of several proteic fraction of the Lactobacillus supernatant. The fractions will be provided by Heinz. The fraction that will retain the activity on gliadin effects on the mTOR pathway will be further fractionated to pick up the range of the activity.
2) We have already found that lactobacillus paracasei supernatant can lower mTOR phosphoryaton and increase LC3 at the same levels as the starvation in CaCo2 cells. What is the element present in the Lactobacillus supernatant that is able to deactivate the m-TOR pathway in CaCo2 cells? The same biological set up as before (point 1) will allow individuate the fraction that can de-phosphorylate mTOR and activate LC3 in CaCo2 cells. This is of particular interest for the industry. In fact, the mTOR pathway is strictly connected with longevity.
3) What activity is left after digestion? This is an important point to assess the future applications of the probiotic. Probiotic supernatant will be digested in an in vitro digestion system already developed by the industry. The system is semi-automated and simulate all the steps of the digestion from mastication/salivation to duodenal enzymatic digestion. The flow trough of the digested Lactobacillus will be tested in our cellular model for the activation of the m-TOR pathway. We will perform the digestion experiments in the industry location.
4) The mechanism of the probiotics effects on mTOR pathway will be studied in a more complex system. We will use organoids from intestinal biopsies to test the effect of the most effective probiotics on the m-TOR pathway both in presence and in absence of P31-43. The organoids are already available in the laboratory and they will be grown in two dimensions on a transwell to separate the apical part

from the basal part of the epithelial cells This will allow us to recreate an intestinal epithelium that can be challenged, and if needed genetically manipulated. The system allows different degrees of complexity from the simple epithelium that can be challenged with the probiotics products and gliadin, to the possibility of adding the different immunological players known to have a role in the pathogenesis of the celiac disease. We will be produce organoids from controls and CD patients at different stage of the disease.

5) Electron microscopy done at Prof Zimmer laboratory in Giessen (Germany) will allow study at the morphological level the effect of the probiotics on intestinal integrity by studying autophagy and cell- cell and cell- substrate interaction in presence and absence of gliadin and probiotics.

Publications

1. *Nanayakkara M, Lania G, Maglio M, Discepolo V, Sarno M, Gaito A, Troncone R, Auricchio S, Auricchio R, Barone MV. An undigested gliadin peptide activates innate immunity and proliferative signaling in enterocytes: the role in celiac disease. Am J Clin Nutr. 2013; 98:1123-35.*

2. *Barone MV, Nanayakkara M, Paolella G, Maglio M, Vitale V, Troiano R, Ribecco MT, Lania G, Zanzi D, Santagata S, Auricchio R, Troncone R, Auricchio S. Gliadin peptide P31-43 localises to endocytic vesicles and interferes with their maturation. PLoS One. 2010; 5(8):e12246.*

3. *Sarno M, Lania G, Cuomo M, Nigro F, Passannanti F, Budelli A, Fasano F, Troncone R, Auricchio S, Barone MV, Nigro R, Nanayakkara M. Lactobacillus paracasei CBA L74 interferes with gliadin peptides entrance in Caco-2 cells. Int J Food Sci Nutr. 2014; 65(8):953-9.*

4. *Labruna G, Nanayakkara M, Pagliuca C, Nunziato M, Iaffaldano L, D'Argenio V, Colicchio R, Budelli AL, Nigro R, Salvatore P, Barone MV, Sacchetti L. Celiac disease-associated Neisseria flavescens decreases mitochondrial respiration in CaCo-2 epithelial cells: Impact of Lactobacillus paracasei CBA L74 on bacterial-induced cellular imbalance. Cell Microbiol. 2019; 1:e13035.*

5. *Calvanese L, Nanayakkara M, Aitoro R, Sanseverino M, Tornesello AL, Falcigno L, D'Auria G, Barone MV. Structural insights on P31-43, a gliadin peptide able to promote an innate but not an adaptive response in celiac disease. J Pept Sci. 2019; 25:e3161.*

6. *Nanayakkara M, Lania G, Maglio M, Auricchio R, De Musis C, Discepolo V, Miele E, Jabri B, Troncone R, Auricchio S, Barone MV. P31-43, an undigested gliadin peptide, mimics and enhances the innate immune response to viruses and interferes with endocytic trafficking: a role in celiac disease. Sci Rep. 2018; 8:10821.*

7. *Lania G, Nanayakkara M, Maglio M, Auricchio R, Porpora M, Conte M, De Matteis MA, Rizzo R, Luini A, Discepolo V, Troncone R, Auricchio S, Barone MV. Constitutive alterations in vesicular trafficking increase the sensitivity of cells from celiac disease patients to gliadin. Commun Biol. 2019; 2:190.*

8. *Gallo M, Nigro F, Passannanti F, Nanayakkara M, Lania G, Parisi F, Salameh D, Budelli A, Barone MV, Nigro R. Effect of pH control during rice fermentation in preventing a gliadin P31-43 entrance in epithelial cells. Int J Food Sci Nutr. 2019; 10:1-9.*

9. *Paolella G, Lepretti M, Martucciello S, Nanayakkara M, Auricchio S, Esposito C, Barone MV, Caputo I. The toxic alpha-gliadin peptide 31-43 enters cells without a surface membrane receptor. Cell Biol Int. 2018; 42:112-120.*

10. *Paolella G, Lepretti M, Barone MV, Nanayakkara M, Di Zenzo M, Sblattero D, Auricchio S, Esposito C, Caputo I. Celiac anti-type 2 transglutaminase antibodies induce differential effects in fibroblasts from celiac disease patients and from healthy subjects. Amino Acids. 2017; 49:541-550.*

External collaborations

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