SENSITIVE CELLULAR BIOASSAYS FOR THE VALIDATION OF T CELL IMMUNOMODULATORY AND GLUTEN DETOXIFYING STRATEGIES FOR TREATMENT OF CELIAC DISEASE

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Background

Celiac disease (CD) is an immune-mediated disorder with trait of autoimmunity, caused by dietary gluten. The pathogenic mechanism responsible of lesions to gut mucosa is a massive cell-mediated immune response to gliadin, the main component of gluten. CD4+ T lymphocytes are central players in the immune response triggered by gluten in celiac patients, as demonstrated in several papers published since the pioneering ones of a Norwegian group on 1993. In the last two decades, T cell lines and clones isolated from the intestinal mucosa of celiac patients, and highly reactive to gluten peptides, have been largely used to explore the inflammatory pathways responsible of CD. Not less important, these T cells represent a sensitive bioassay for the in vitro validation of immunomodulatory and gluten detoxifying strategies, a necessary step before the in vivo clinical studies.
An additional approach to examine the T cell mediated response to gluten is the short-term oral wheat challenge, basically an in vivo procedure that allows to monitor the gluten-specific T cells in peripheral blood of celiac patients in disease remission, after a medical controlled consumption of a low amount of wheat food. In the last years, thanks to these two in vitro and in vivo tools, great step forwards were reached in the identification of the repertoire of pathogenic gluten peptides, and in the extensive characterization of phenotype and function of gluten-reactive T cells.
Recently, these experimental cellular systems also provided a great support to studies aiming to identify novel therapeutic drugs for CD, as well as to validate biochemical/enzymatic strategies to detoxify gluten containing cereals.

Main achievements

A diet completely deprived of gluten is, currently, the only efficacious treatment for CD. Some limitations of gluten-free therapy, particularly due to the poor compliance for some patients during to social events and travelling, have encouraged the searching of alternative strategies aimed to improve the life quality of patients. We have exploited the use of celiac T cell-based bioassays to evaluate the efficacy of novel therapeutic strategies, as well as the immunotoxicity of ancient wheat species, as the Triticum monococcum species, in order to find wheat crops with null or low content of gluten toxic peptides. To date, the most promising strategies for the treatment of CD are based on enzymatic approaches aiming to degrade gluten fragments escaping the digestion of gastrointestinal proteases, thus destroying their immune-stimulatory sequences. The "oral enzyme therapy", based on proteases able to cleave the Q-P bonds in gluten proteins (named glutenases), has been proposed to promote complete digestion of disease-inducing gluten peptides in gastric conditions. We have demonstrated, by using celiac T cell assays, the ability of a novel engineered glutenase, Kuma030, to efficiently degrade immunogenic gluten peptides, resulting in a strong reduction of activity on celiac T cells of whole gliadins. In more details, Kuma030-treated gliadins, at specific enzyme: gliadin concentrations ratios, resulted in marked reduction of capability to stimulate the INF-γ production by celiac T cells (Figure 1).

In line with these results, we took advantage of these T cell assays to assess the efficacy of the pre-treatment of wheat flour with a mixture of selected sourdough, lactobacilli and fungal proteases to detoxify gluten proteins. The in vitro cellular assays confirmed the efficacy of wheat flour fermentation with acidic microorganisms to reduce the immune-reactivity of gluten. As shown in Figure 2, gliadin extracted from treated wheat flour was not able to activate the INF-γ production, if compared to untreated gliadin. In a next study, we confirmed these findings with the short-term oral challenge in CD patients that consumed bread made with treated wheat flour or with control toxic wheat flour (Figure 3).

As stated above, in the recent time, we have focused our efforts to the identification of wheat species with a null or very low content of toxic gluten sequences, in order to find cultivars suitable for disease prevention in subjects at high genetic risk of CD. In particular, we have dissected the immunological properties of the gluten from the ancient wheat species T. monococcum, by an extensive proteomic, immunoenzymatic and T cell-based analyses. Unequivocally, we demonstrated the capability of gluten from two cultivars of T. monococcum to stimulate T cells from celiac intestinal mucosa, thus not suitable for those people with a CD diagnoses. Interestingly, by a further investigation, we found that, after an in vitro digestion mimicking the gastrointestinal hydrolyses, gliadin from T. monococcum wheat retained a reduced

immunogenicity compared to that of common wheat species (T. aestivum). Our results demonstrated that monococcum gluten immunogenic peptides are extensively degraded by gastrointestinal proteases, thus suggesting a marked digestibility of this ancient wheat variety compared to common bread wheat.

Future perspectives

Currently, our research is focused in different projects aiming to take advantages of our bioassays, based on gliadin-reactive T cells, for their validate use: 1) to search naturally non-toxic, or less toxic, cereals for CD treatment or prevention; 2) to identify new strategies to detoxify wheat gluten; 3) to validate novel immunomodulatory strategies that aim to inhibit the intestinal inflammatory reaction in gluten-exposed CD patients. These research projects are done in collaboration with national and international academic institutes or private biotech companies.

Publications

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Figure 1. Dose curve INF-γ production in intestinal T cell lines from 3 celiac patients in response to gliadin treated with the glutenase Kuma030. Gliadin was incubated with Kuma030 at different enzyme concentration for 60 min at pH 4.0 at 37 °C. INF-γ was measured in cell supernatant by standard sandwich ELISA.

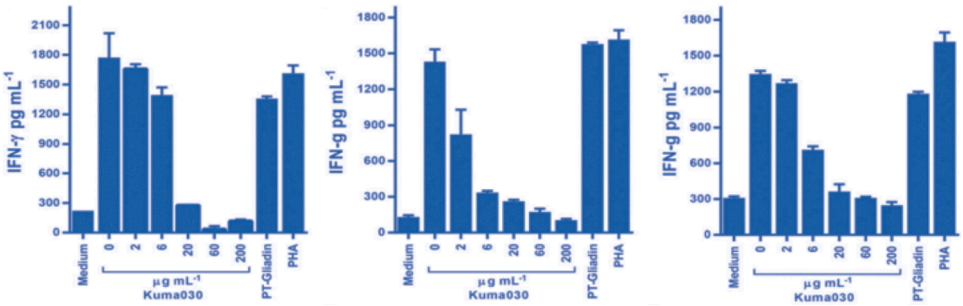
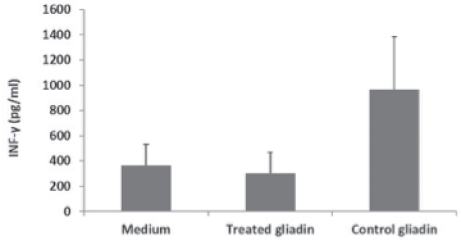


Figure 2. A) Average of INF-γ production in in vitro stimulated intestinal T cell lines from three coeliac patients in response to untreated control gliadins and gliadin treated with the mixture of sourdough, lactobacilli and fungal proteases. B) INF-γ responses of individual celiac patient. INF-γ was measured in cell supernatant by standard sandwich ELISA.

A)



B)

	Medium	Treated gliadin	Control gliadin
CD1	545.3	480.3	1013.6
CD2	220.8	149.7	1357.8
CD3	329.6	277.9	525.9

Figure 3. Gliadin-specific T cells circulating in peripheral blood of celiac patients underwent a brief consumption of bread made with natural wheat flour (control gliadin) or wheat flour treated with lactobacilli and fungal proteases (treated gliadin). The INF-γ secreting cells were detected in peripheral blood by ELISPOT assay soon before (day 0) and 6 days after (day 6) the 3 days of wheat consumption.