Intestinal T-cell based assays: from basic science to translational application for treatment of celiac disease

Celiac disease (CD) is an immune-mediated enteropathy, caused by gluten proteins characterized by an increased prevalence worldwide in the last decades. CD4+ T cells are central players in the inflammatory reaction to dietary gluten. In the recent time, 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 (1). Not less important, these T cell cultures represent a sensitive bioassay for the *in vitro* validation of immunomodulatory and gluten detoxifying strategies, a necessary step before the *in vivo* clinical studies (2).

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 young patients. 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 based bioassays, the ability of a novel glutenase, the endoprotease-40 (E40) of microbial origin, to efficiently degrade immunogenic gluten peptides, resulting in a strong reduction of bioactivity on celiac T cells of whole glutens (2).

An additional approach to examine the T-cell mediated response to gluten is the short-term oral challenge, basically an *in vivo* procedure that allows to monitor the gluten-specific T cells in peripheral blood of CD patients in disease remission, after a medical controlled consumption of a low amount of wheat food (3). This bioassay has been successfully applied to the search of wheat species with a null, or very low, content of toxic gluten sequences, suitable for disease prevention in subjects at high genetic risk of CD (4). In particular, we have dissected the immunological properties of gluten from monococcum, an ancient wheat cultivar, by an extensive proteomic, immunoenzymatic and T cell-based analyses. Unequivocally, we demonstrated the capability of gluten from two monococcum cultivars 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 hydrolys, monococcum gluten proteins retained a reduced immunogenicity compared to that of common wheat species (such as soft wheat). Our results demonstrated that gluten peptides from ancient crops are extensively degraded by gastrointestinal proteases, thus suggesting a marked digestibility of this ancient wheat variety compared to common bread wheat (4).

Currently, our research is focused in different projects aiming to take advantages of our sensitive bioassays, based on gluten-reactive T cells, as listed:

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.
References:


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