IMMUNOGENICITY OF MONOCOCCUM

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Background

The spectrum of gluten-related disorders (GRD) includes celiac disease (CD), dermatitis herpetiformis, gluten ataxia, wheat allergy (WA), and non-celiac gluten sensitivity (NCGS). Preliminary data observed in NCGS would suggest that innate rather than adaptive immunity has a prominent pathogenic role. Our group has shown an increased expression of toll-like receptor (TLR) 2 and claudin (CLDN) 4 in NCGS subjects [1]. Additional compelling evidence for the role of innate immunity in NCGS came from other groups, showing an increased production of TNF-α and IL-8 [2,3]. In CD an immune response to gluten is mediated by the innate and adaptive immune branches. Therefore, a cereal suitable for a CD diet should be low in both classes of peptides. Diploid wheat species are among the suitable candidates because of their low tendency to activate intestinal T cell responses in CD patients [4,5]. Our preliminary study on two accessions of ancient wheat Triticum monococcum, Monlis and ID331, shows that Monlis seems to be able to activate both innate and adaptive immune responses, whereas ID331 seemed to activate only an adaptive immune response [6]. Subsequently, we demonstrated that gliadin proteins of Monlis and ID331 are sufficiently different from those of common wheat and have lower immune toxicity following in vitro simulation of human digestion [7]. α-amylase/trypsin inhibitors (ATIs) are another category of wheat proteins identified as strong activators of innate immune responses. A recent study [8] found that ATIs engage TLR-4 and release IL-8 in myeloid cells of both patients with CD and controls, as is expected for innate immune triggers. Moreover, the authors also showed that the addition of exogenous ATIs to the organ culture of jejunal biopsies from treated CD induced an increase in IL-8 mRNA levels. Interestingly, it was found that modern wheat contains high concentrations of ATIs compared with ancient diploid wheat [9].

These data reinforce the hypothesis of the potential use of old cultivars as potential dietary options for patients suffering from CD or other gluten-related disorders.
The aim of this project, was to compare the content and the immunological properties of ATIs obtained from ancient diploid T. monococcum wheat (Monlis and Norberto-ID331) and common hexaploidy wheat (Triticum aestivum).

References

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Main achievements

In the last few years we obtained important results regards the studies of the new clinical entity named non-celiac gluten sensitivity and on the potential use of old wheat crops for the diet of individuals with gluten related disorders. In particular, we have shown:

- by the 2015 Salerno Expert's Criteria, that the term NCGS is used to describe the clinical state of individuals who develop both intestinal and extraintestinal symptoms when they consume gluten-containing foods and feel better on a gluten-free diet (GFD) but do not have CD or a WA;
- that NCGS, albeit gluten-induced, is different from CD not only with respect to the genetic makeup and clinical and functional parameters, but also with respect to the

nature of the immune response. Our findings also suggest that two subgroups of CD, IL-17-dependent and IL-17-independent, may be identified based on differential mucosal expression of this cytokine.

- an increased expression of toll-like receptor (TLR) 2 and claudin (CLDN) 4 in NCGS subjects suggesting that innate rather than adaptive immunity has a prominent role in in the pathogenesis of NCGS;
- that Monlis in CD, seemed to be able to activate both innate and adaptive immune responses, whereas ID331 seemed to activate only an adaptive immune response;
- that gliadin proteins of Monlis and ID331 are sufficiently different from those of common wheat and have lower immune toxicity following in vitro simulation of human digestion.

Future perspectives

ATIs will be purified from diploid T. monococcum wheat flours, (Monlis and Norberto-ID331 cultivars), and hexaploidy T. aestivum wheat flours (Sagittario cultivar) and characterized by proteomic analysis. The ATIs will be digested with pepsin-chymotrypsin (PC) enzymes and assayed on jejunal biopsies from treated CD patients and NCGS patients, to evaluate the expression of IL-8 and TNF-α, by qRT-PCR. The ATIs immunogenicity will be also assayed on gut-derived T cell lines (TCLs), previously established from small intestinal biopsies obtained from celiac patients and IFN-γ production measured by ELISA. Our study will provide more insights on the nature of T. monococcum ATIs in particular we aim to demonstrate that diploid wheat species are substantially different from those of hexaploid modern wheats for their reduced ability to trigger the innate branch of immunity. Overall, these findings could indicate that T. monococcum retains a lower toxicity for subjects suffering from gluten-related disorders and may be used as potential dietary options for such patients. To address this challenge, future clinical trials will be performed. In particular, a double-blind, gluten-controlled clinical trial to evaluate the safety of the gluten from monococcum wheat flour in NCGS patients, will be performed.

Publications

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