

# Gluten Sensitivity is Associated to Activation of The Innate But Not Adaptive Immune Response to Gluten Exposure

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**Background:** The immune responsiveness to gluten-containing grains represents a complex process whose establishment and maintenance are not completely elucidated. There are cases of gluten reaction defined as gluten sensitivity (GS) in which neither an allergic (wheat allergy) nor an autoimmune [celiac disease (CD)] mechanism can be advocated. Recent evidences suggest that early changes in intestinal permeability (IP) and activation of both innate and adaptive immune responses are involved in CD pathogenesis. Conversely, no data are currently available on the mechanisms leading to GS.

**Aims:** To investigate the changes in IP, TJ protein genes expression, and innate and adaptive immune responses in GS.

**Methods:** After obtaining informed consent, biopsy samples were obtained from 28 GS patients, 53 pts with active CD, 16 patients with CD in remission, and 37 healthy controls (age range: 5 years -50 years). Quantitative gene expression of TJ proteins Claudin (CL) 1, CL2, CL3, CL4, ZO-1, TLR1, TLR2 and TLR4, FOXP3, and TGF- $\beta$  were performed by Real-time PCR. IP was evaluated by means of the lactulose/mannitol test (LA/MA). The numbers of intraepithelial lymphocytes (IELs) were detected with CD3 and TCR- $\gamma\delta$  immunostaining and examined by counting the peroxidase stained cells. ELISA analysis of IL6, IL8, TNF $\alpha$ , was conducted on PBMC of all patients.

**Results:** Intestinal CL3 and CL4 expressions were significantly increased in GS subjects compared to CD patients ( $p < 0.01$ ), while no changes in other TJ protein genes expression were detected. In GS patients, these changes were associated to a lower IP ( $0.010 \pm 0.008$ ) that inversely correlated to CL4 gene expression ( $r = -0.6318$ ;  $p < 0.05$ ) compared to healthy controls ( $0.018 \pm 0.009$ ). Conversely, in CD patients an over-expression of CL2 was observed that was associated to increased IP ( $0.053 \pm 0.048$ ) and that returned to baseline following implementation of a gluten free diet. Interestingly, immunohistochemical examination of biopsy specimen showed a lower number of CD3+IELs in GS pts compared to active CD pts (27.25/100 and 36.3/100 enterocytes respectively) that correlate with a MARSH 0-1 lesions, with no changes in TCR- $\gamma\delta$  IELs. In a subgroup of GS pts, intestinal TLR1 and TLR2 expression was increased and these changes were paralleled by increased production by PBMC of cytokines related to the innate but not adaptive immune responses. **Conclusions:** Compared to CD patients, GS subjects showed normal IP and activation of the innate but not adaptive immune responses. These changes cause only minimal gut inflammation, suggesting that in GS lack of adaptive immune response involvement prevent the autoimmune gut insult typical of CD.