

# Gluten Sensitivity versus Celiac Disease: Differences in Intestinal Permeability, Tight Junction Proteins Expression, and Immunological Mechanisms

Update: Differential Mucosal IL-17 Expression in Two Gliadin-induced Disorders: Gluten Sensitivity and the Autoimmune Enteropathy Celiac Disease

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## Gluten Sensitivity versus Celiac Disease: Differences in Intestinal Permeability, Tight Junction Proteins Expression, and Immunological Mechanisms

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. The specific aim of our research has been to identify possible parameters [ intestinal barrier dysfunction (IP), immunological and molecules expression (tight junctions protein)] for better differentiate GS from CD conditions in humans. Particularly, Claudin (CL) 1, CL2, CL3, CL4, ZO-1, and TLR1, TLR2 and TLR4, FOXP3, TGF- $\beta$  and IL 17A gene expression were performed by Real-time PCR. IP was evaluated by means of the lactulose/mannitol test. The number of CD3<sup>+</sup> and TCR- $\gamma\delta$  lymphocytes, and the proportion of CD3<sup>+</sup> cells coexpressing the Th17 marker, CCR6, were examined by *in situ* small intestinal immunohistochemistry. ELISA analysis of IL6, IL8, TNF $\alpha$  was conducted on PBMC of all patients. Here we report that CL3 and CL4 expressions were significantly increased in GS subjects compared to CD patients ( $p < 0.01$ ). 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 ( $0.053 \pm 0.048$ ). In a subgroup of GS pts, intestinal TLR1 and TLR2 expression was increased and these changes were associated to increased production of cytokines related to innate but not adaptive immune responses. Interestingly, immunohistochemical examination of biopsy specimen showed a lower number of CD3<sup>+</sup>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. This evidence was associated to an elevated IL 17 expression in a subgroup of CD but not in GS patients and was paralleled by a trend toward increased proportions of CD3<sup>+</sup>CCR6<sup>+</sup> cells in intestinal mucosal specimens from these subjects. In conclusions our data showed that compared to CD patients, GS subjects showed normal IP and activation of the innate but not TH1 and TH17 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.

# Background:

Reaction to gluten can involve an allergic (wheat allergy) or an autoimmune [celiac disease (CD)] mechanism.

However, there are cases of gluten reaction in which neither mechanism is involved. These cases are defined as gluten sensitivity (GS).

Recent evidences suggest that early changes in intestinal permeability (IP) and activation of the innate immune system through Toll Like Receptor (TLR) signaling pathway may both be involved in the early steps of CD pathogenesis.

Conversely, no data are currently available on the role of intestinal barrier dysfunction in the pathogenesis of GS.

# Aims:

- . To investigate the changes in IP and TJ protein genes expression in GS patients;
- . To establish whether these changes are related to activation of the innate and/or adaptive immune response.

# Materials and Methods:

- Biopsy samples were obtained from 26 GS patients, 30 patients with active CD, 6 patients with CD in remission, and 14 healthy controls.
- Quantitative gene expression of tight junctions proteins Claudin (CL) 1, CL2, CL3, CL4, ZO-1, Occludin and of TLR1, TLR2 and TLR4 was performed by Real-time PCR.
- IP was evaluated by means of the lactulose/mannitol test (LA/MA).
- The degree of intestinal mucosal damage was evaluated according to the Marsh classification.
- The numbers of IELs were examined using CD3, TCR- $\gamma\delta$  and CCR6 immunostaining.

# Materials and Methods:

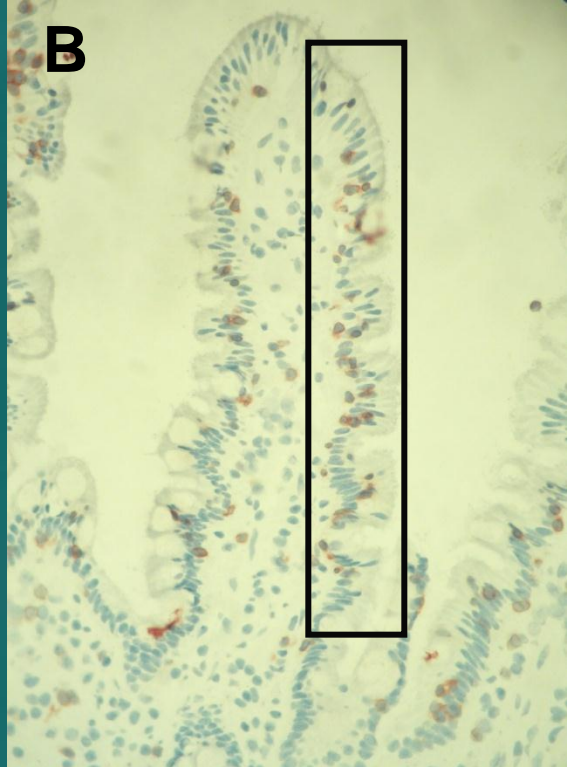
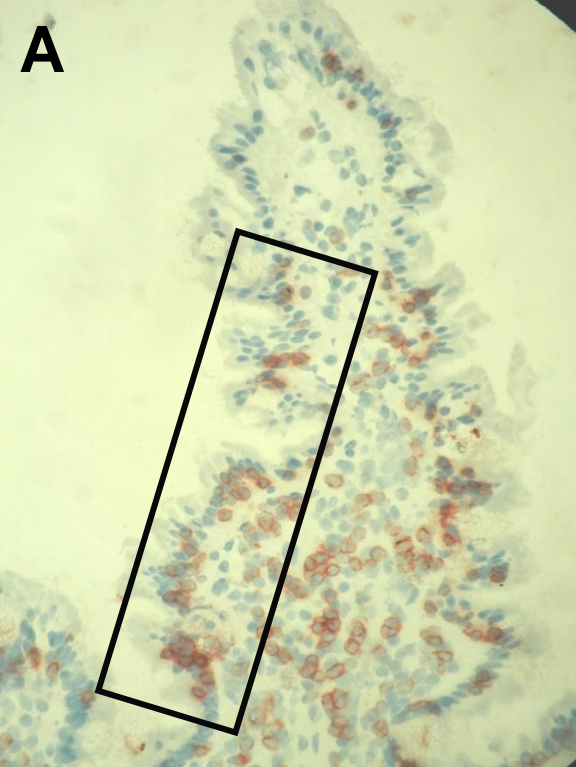
GS inclusions criteria:

- Gastrointestinal or extra-intestinal symptoms mimicking CD;
- Negative CD serology;
- Negative response for atopy patch test (APT) to wheat and prick test.

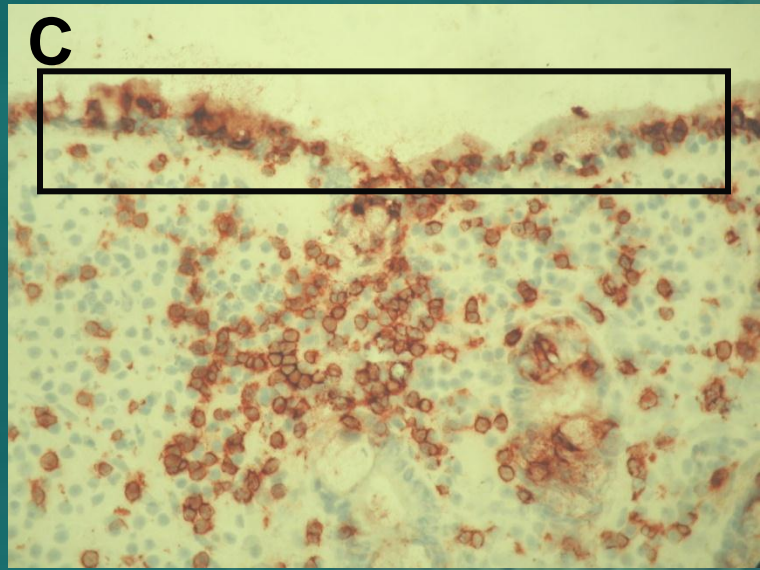
# Results:

## Clinical and laboratory characteristics of the study subjects

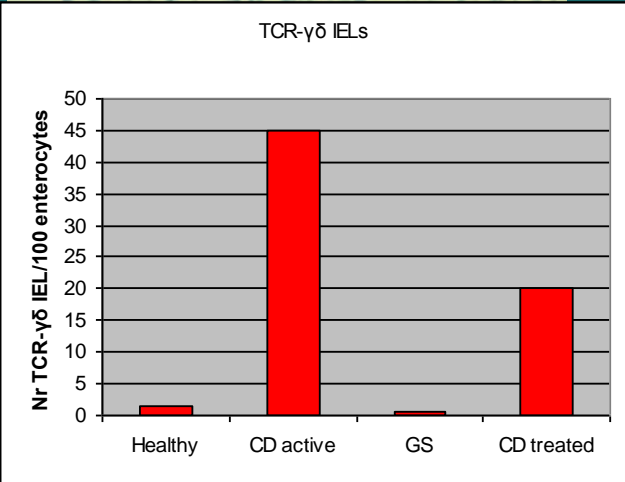
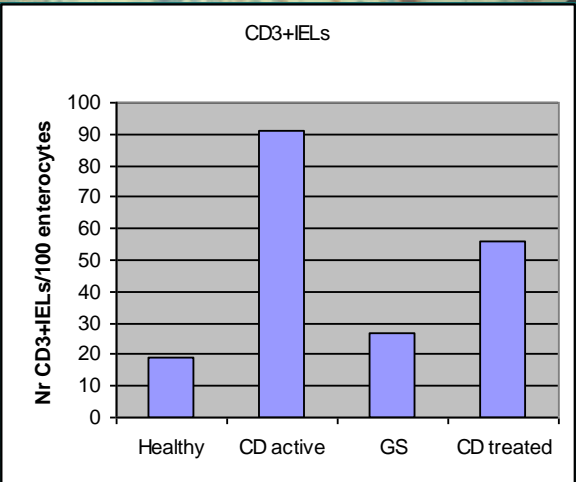
	Healthy	CD	GS
Age of diagnosis (mean ± SE)	29.1 ± 5.1	35.2 ± 3.3	28.9 ± 4.4
Sex (F/M)	20/16	15/36	18/10
Intestinal Symptoms	Dyspepsia	<ul style="list-style-type: none"> <li>-Chronic diarrhea</li> <li>-Abdominal pain</li> <li>-Weight fluctuation</li> <li>- Weakness</li> <li>- Smelling, fatty stool</li> </ul>	<ul style="list-style-type: none"> <li>-Diarrhea</li> <li>- Abdominal pain</li> <li>-Weight fluctuation</li> <li>-Gas</li> </ul>
Extra-intestinalSymptoms	None	<ul style="list-style-type: none"> <li>-Bone or joint pain</li> <li>- Osteoporosis</li> <li>- Behavioral changes</li> <li>- Tingling leg numbness</li> <li>- Muscle cramps</li> <li>- Missed menstrual periods</li> <li>- Infertility</li> <li>- Recurrent miscarriage</li> <li>- Delayed growth</li> <li>- Tooth discoloration</li> <li>-Unexplained anemia</li> </ul>	<ul style="list-style-type: none"> <li>-Bone or joint pain</li> <li>-Osteoporosis</li> <li>- Leg numbness</li> <li>-Muscle cramps</li> <li>-Unexplained anemia</li> <li>- Glossitis</li> <li>-Thyroiditis</li> </ul>
EMA	0%	Positive (92.3%)	Negative
AGA	0%	Positive (53.8%)	Positive (45.5%)
†TG	0%	Positive (84.6%)	Negative
HLA DQ2/DQ8	28.6%	92.3%)	36.4%)
PRICK/ RAST/ PRIST test	Not tested	Negative	Negative
LA/MA test	Normal (< 0.030)	Altered (> 0.030)	Normal (< 0.030)



Results : Duodenal IHC  
CD3+ IELs



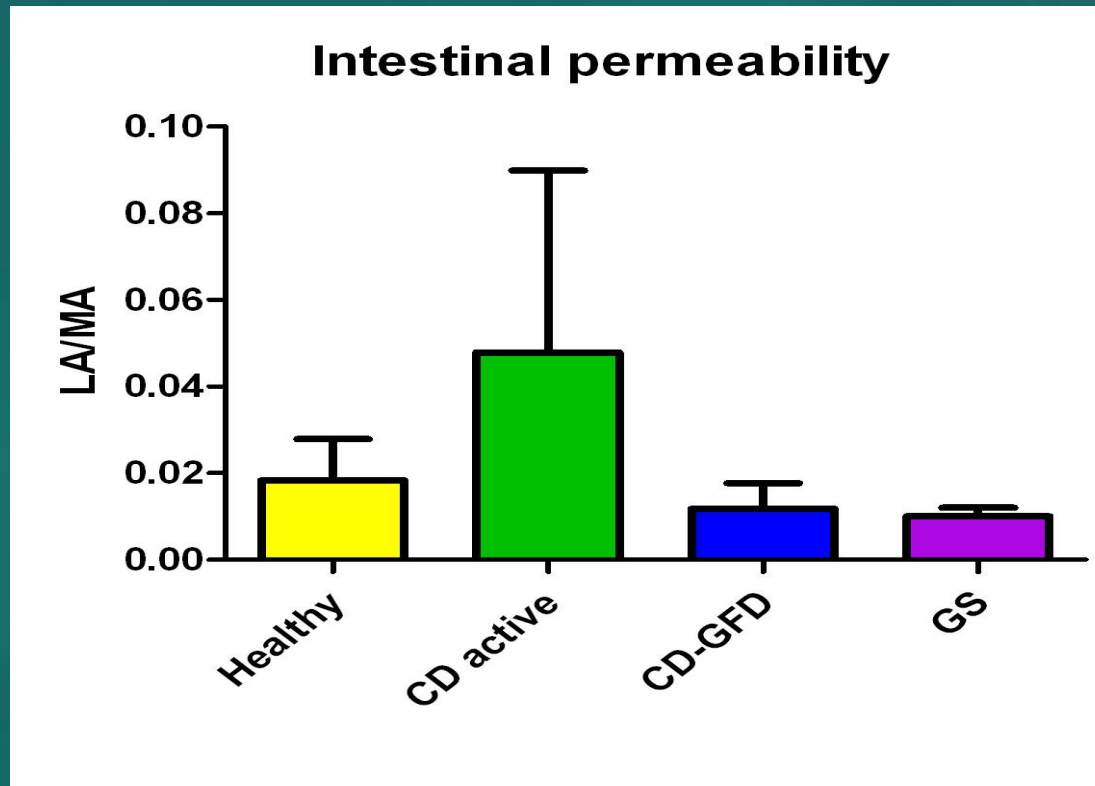
40 X



A = Gluten Sensitive  
B = Controls  
C = CD Active

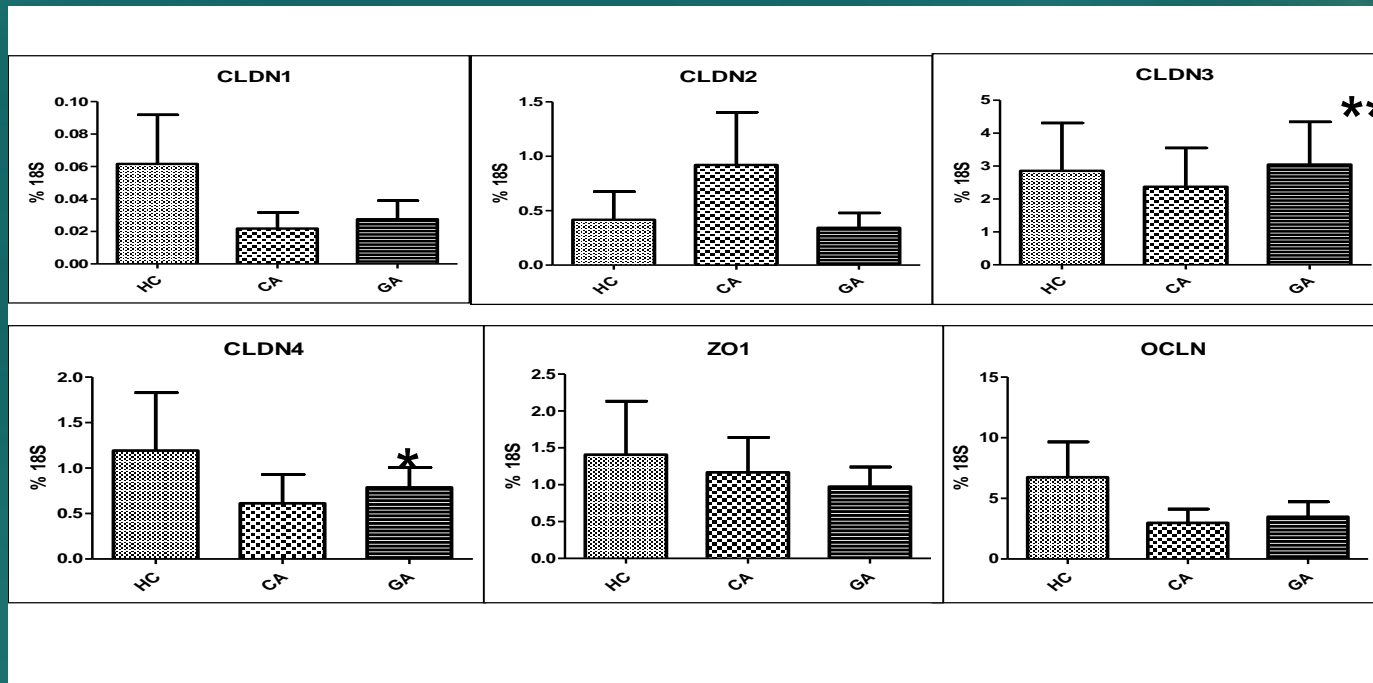
# Results:

## Change in Intestinal Permeability in GS and CD patients



In GS patients IP ( $0.014 \pm 0.015$ ) was similar to that detected in healthy controls ( $0.019 \pm 0.018$ ). Conversely, in CD the increased expression of CL2 was associated to an increase in IP ( $0.052 \pm 0.048$ ). In CD patients in remission, IP ( $0.014 \pm 0.004$ ) returned to normal levels.

# Results: Intercellular TJ expression CD



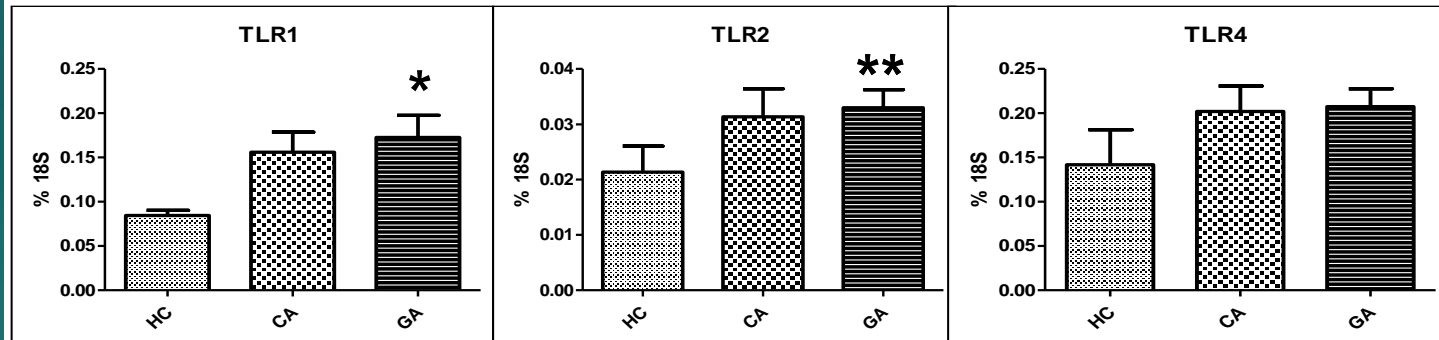
CL3 and CL4 expressions were significantly increased in GS subjects compared to CD patients ( $p < 0.01$ ), while no changes in other TJ protein genes expression were detected. Conversely, in CD patients an over-expression of CL2 was observed that was associated to increased IP and that returned to baseline following a gluten free diet.

\*, \*\*  $P < 0.01$  GS vs Celiac active pts

\*  $r$  (IP vs CL4) = 0,6318;  $p = 0,05$

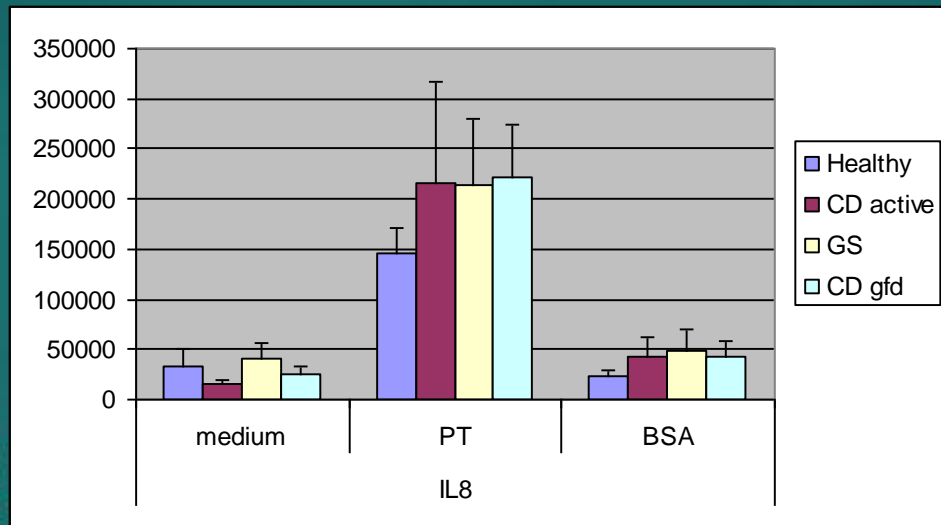
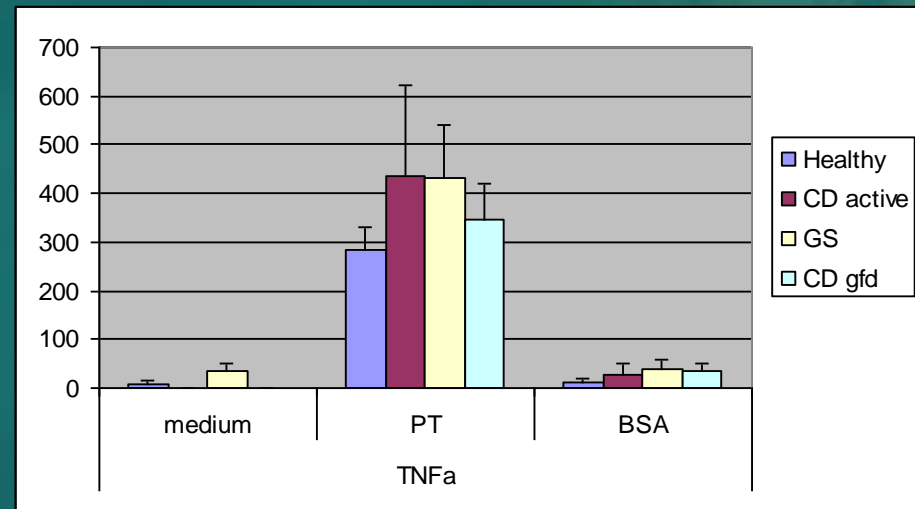
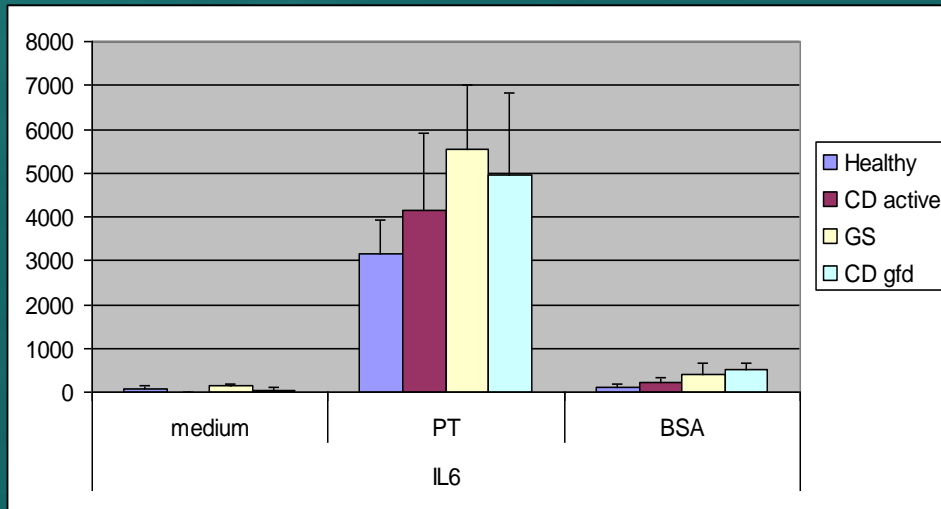
# Results:

## TLR signaling pathway involvement



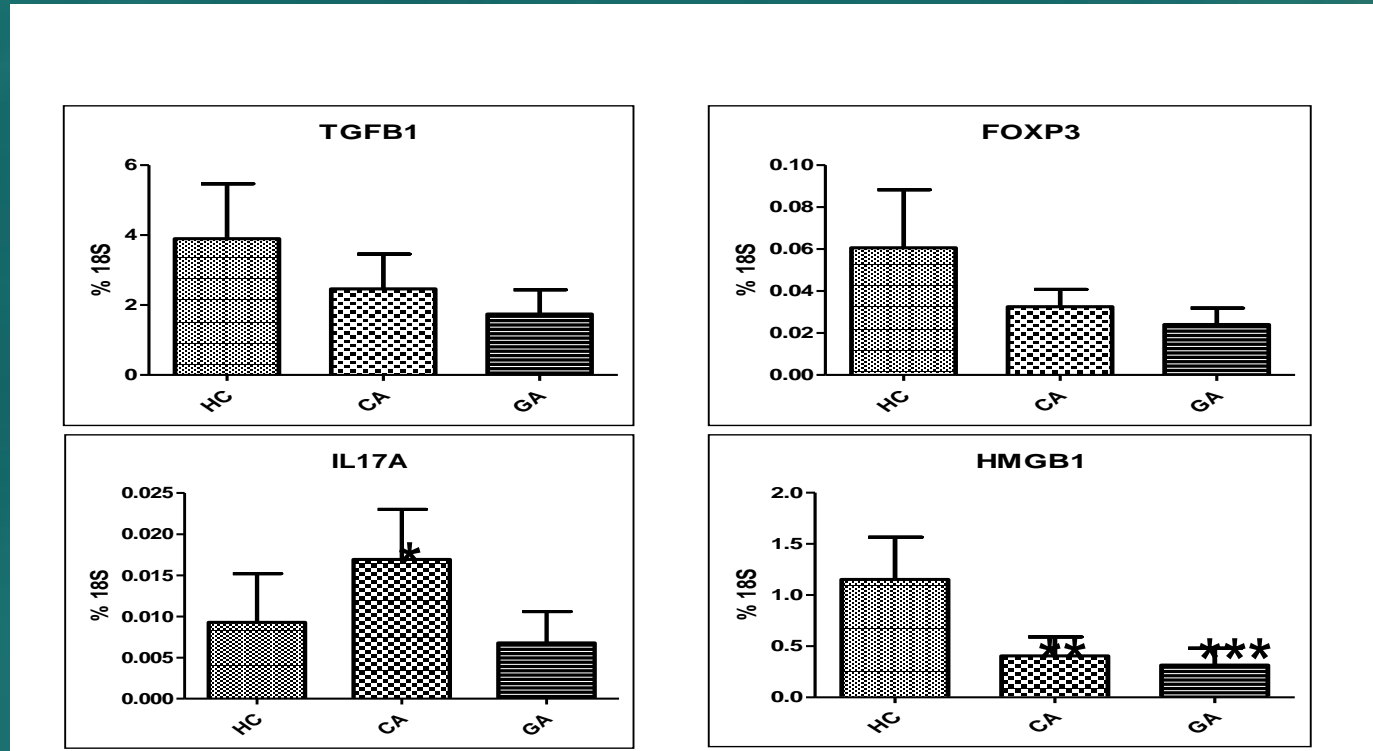
TLR1 and TLR2, but not TLR4 resulted significantly increased in GS  $^{*}.$  $^{**}$ ( $p < 0,05$ ) respect to normal controls, while CD showed only a not significant increase of TLR1

# Results: Cytokines expression in cultured PBMC



PBMC released IL6, IL8 and TNFa in all groups after stimulation with pt-Gliadin.

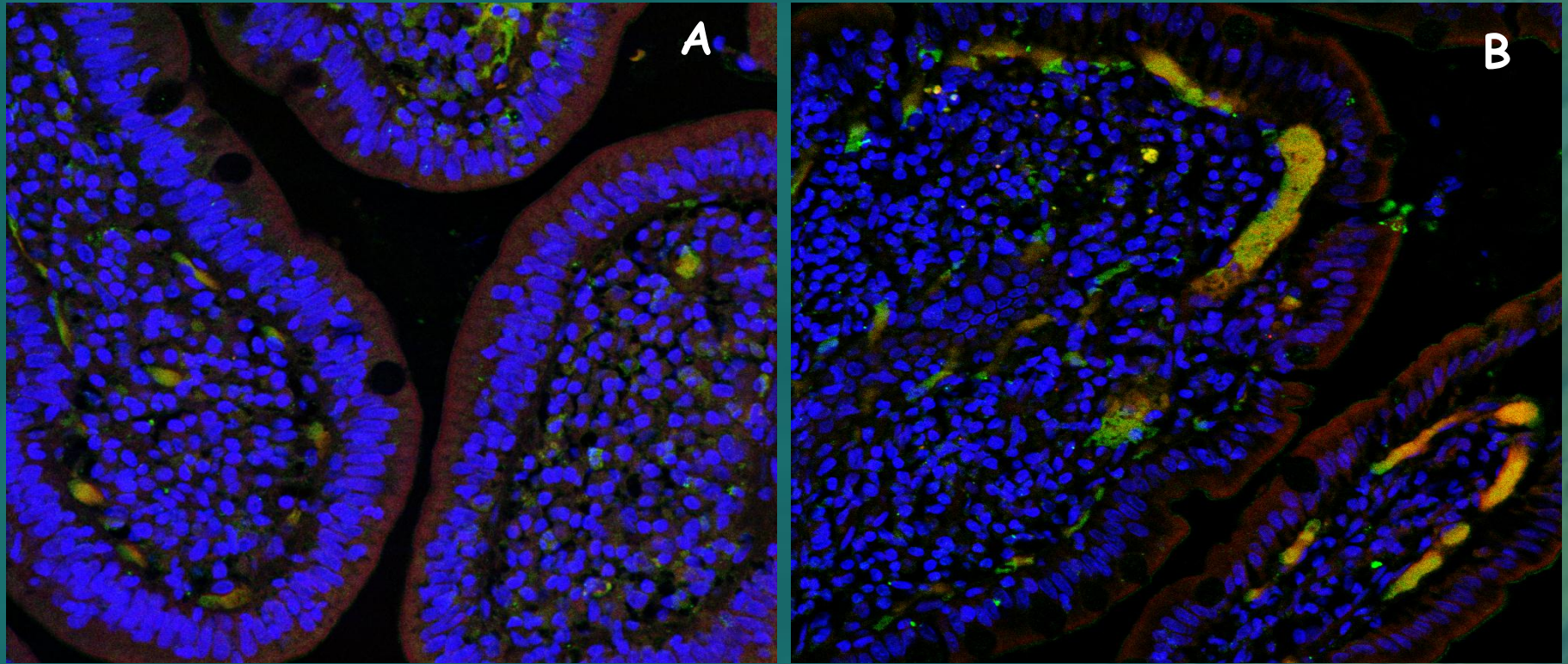
# Results: regulatory molecules gene expression



Celiac disease (CD) patients showed a decreased expression of FOXP3 that was associated to a significantly increased of IL 17 expression compared to healthy controls (HC) and gluten sensitive (GS) patients, while HMGB1 was significantly decreased in both GS and CD pts.

\*  $P=0,04$  CD vs C \*\* ,\*\*\*  $P<0,05$  CD and Gs vs C

# Immunofluorescence staining of mucosal CD3 and CCR6 in GS and CD



40 X

A= Gluten Sensitive

B= CD active

*Sapone A. et al, Int. Arch. of Allergy and Immunology in press*

# Conclusions:

- Compared to CD patients, GS subjects showed normal IP.
- Up-regulation of CL4 in GS patients is statistically significant and inversely correlated to IP.
- GS patients showed an increase in CD3+ IEL but no tissue damage.
- The significant over expression of TLR 1 and TLR2 in GS but not in CD may reflect the activation of the innate immune system in this condition.
- PBMC derived cytokines expression is similarly elicited by gliadin in both CD and GS.
- IL 17 activation pathway only in CD may suggest that in GS the response to gluten exposure is limited to activation of the innate immune system without involvement of the adaptive immune response.
- **Gluten Sensitivity** showing a peculiar TJ proteins pattern, confirms to be different from celiac disease.