

Activation of Innate and not Adaptive Immune system in Gluten Sensitivity

Update: Differential mucosal IL-17 expression in gluten sensitivity
and the autoimmune enteropathy celiac disease

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ACTIVATION OF INNATE AND NOT ADAPTIVE IMMUNE SYSTEM IN GLUTEN SENSITIVITY

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. Aim was to investigate the changes in IP, TJ protein genes expression, and innate and adaptive immune responses in GS. 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). Claudin (CL) 1, CL2, CL3, CL4, ZO-1, and TLR1, TLR2 and TLR4, FOXP3, and TGF- β gene expression were performed by Real-time PCR. IP was evaluated by means of the lactulose/mannitol test (LA/MA). The number of intraepithelial lymphocytes (IELs) was detected with CD3 and TCR- $\gamma\delta$ immunostaining and examined by counting the peroxidase stained cells. ELISA analysis of IL6, IL8, TNF α , was conducted on PBMC of all patients. 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 ± 0.008) that inversely correlated to CL4 gene expression ($r = -0.6318$; $p < 0.05$) compared to healthy controls (0.018 ± 0.009). Conversely, in CD patients an over-expression of CL2 was observed that was associated to increased IP (0.053 ± 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 correlated with 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 associated to an increased production of cytokines related to the innate but not adaptive immune responses. 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.

Background

The evolution of human race did not plan for the advent and exposure to wheat gluten.

The “engineering” of gluten-containing grains 10,000 years ago represented a “mistake of evolution” that created the conditions for human diseases related to gluten exposure.

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 and TCR- $\gamma\delta$ immunostaining.

Materials and Methods:

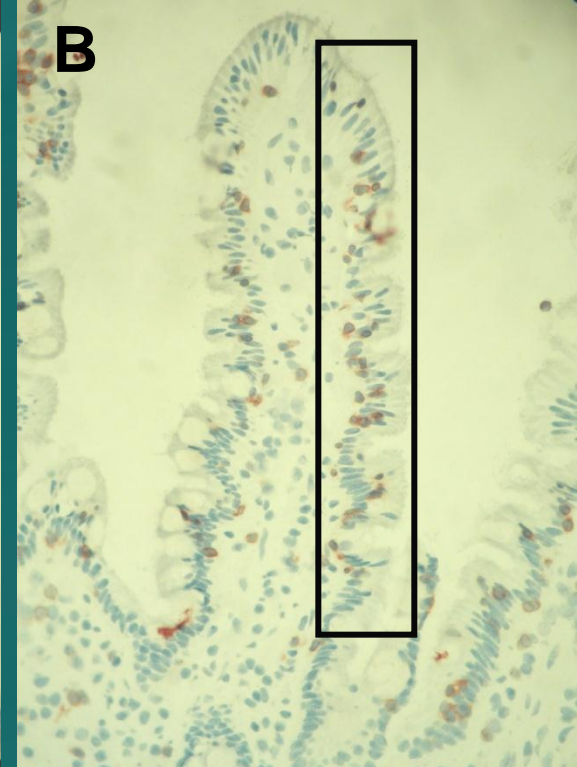
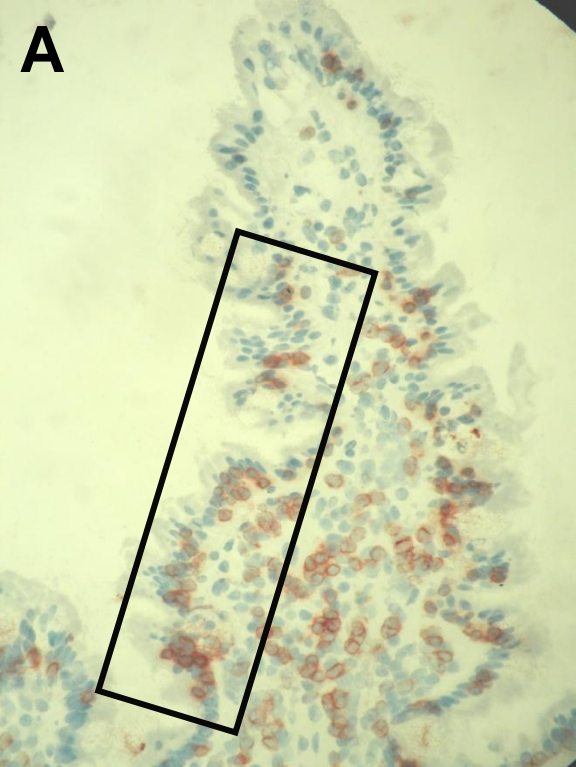
GS inclusions criteria:

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

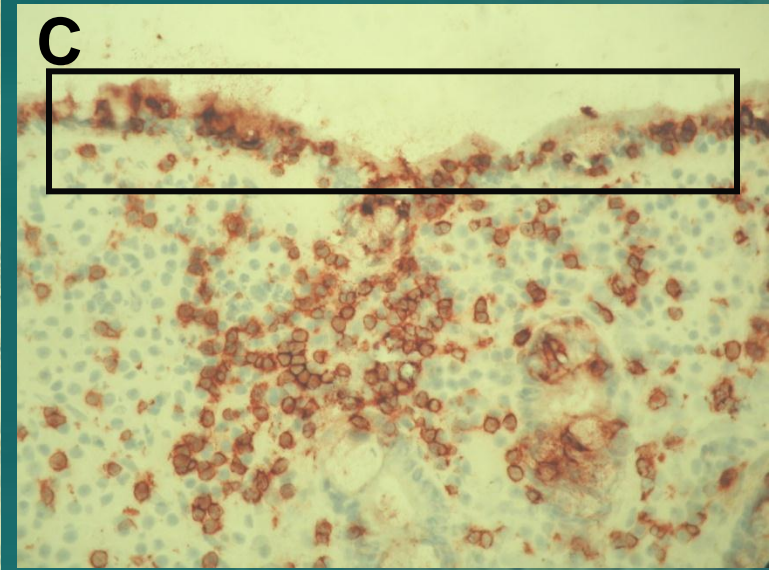
Results:

Clinical and laboratory characteristics of the study subjects

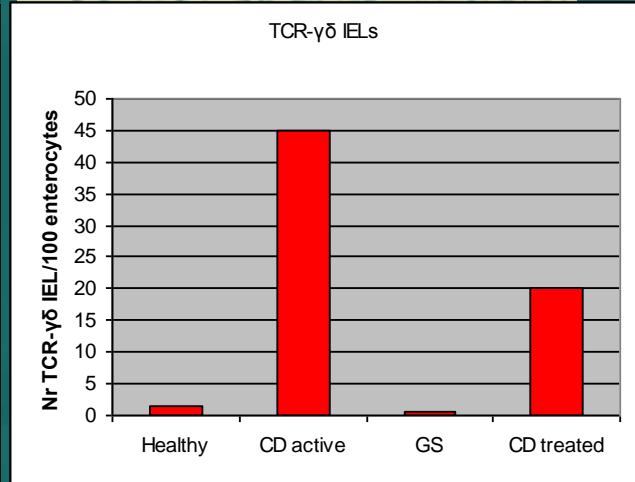
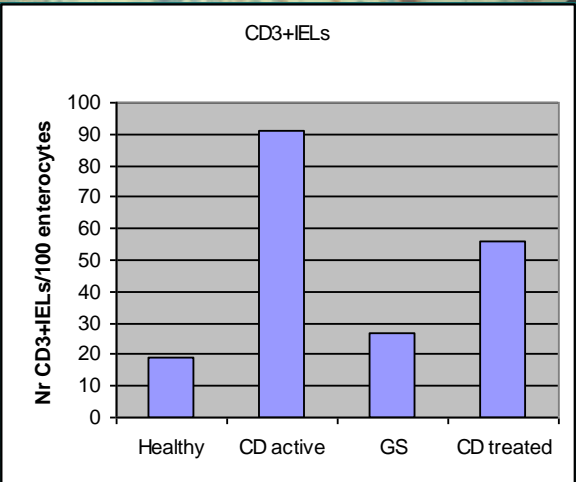
	Healthy	CD	GS
Age of diagnosis (mean ± SE)	29.1 ± 5.1	35.2 ± 3.3	28.9 ± 4.4
Sex (F/M)	20/16	15/36	18/10
Intestinal Symptoms	Dyspepsia	<ul style="list-style-type: none"> -Chronic diarrhea -Abdominal pain -Weight fluctuation - Weakness - Smelling, fatty stool 	<ul style="list-style-type: none"> -Diarrhea - Abdominal pain -Weight fluctuation -Gas
Extra-intestinalSymptoms	None	<ul style="list-style-type: none"> -Bone or joint pain - Osteoporosis - Behavioral changes - Tingling leg numbness - Muscle cramps - Missed menstrual periods - Infertility - Recurrent miscarriage - Delayed growth - Tooth discoloration -Unexplained anemia 	<ul style="list-style-type: none"> -Bone or joint pain -Osteoporosis - Leg numbness -Muscle cramps -Unexplained anemia - Glossitis -Thyroiditis
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



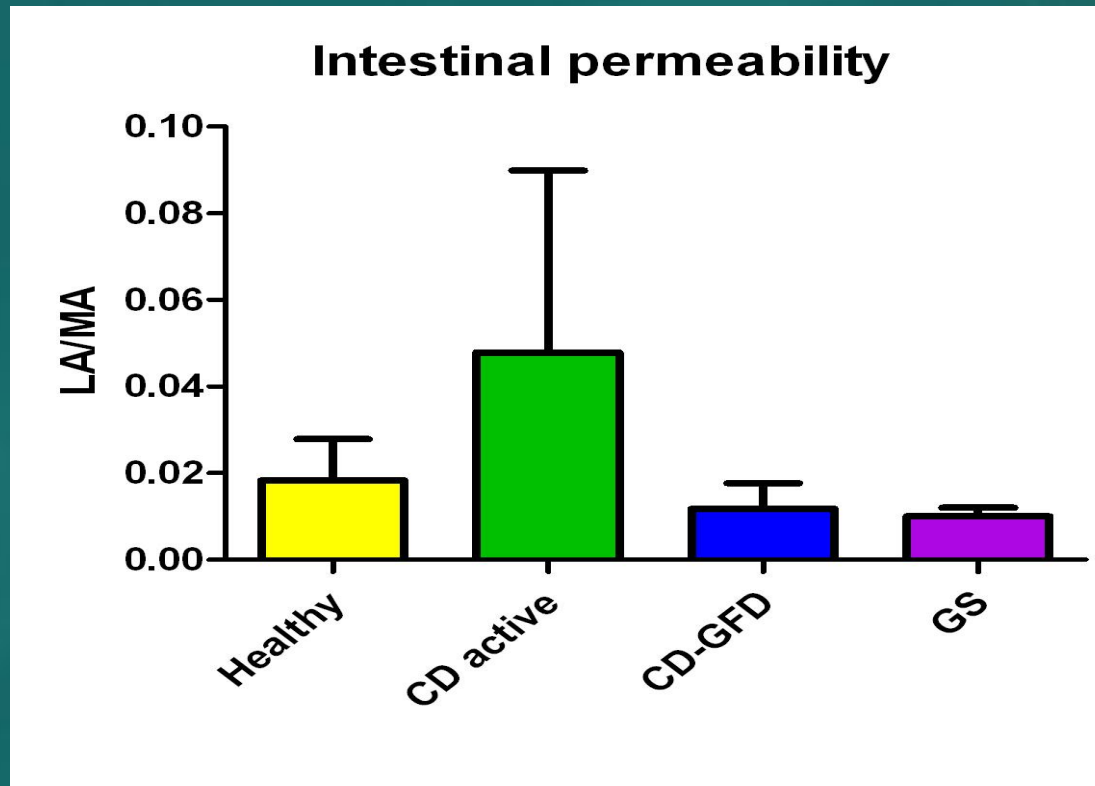
40 X



A = Gluten Sensitive
B = Controls
C = CD Active

Results:

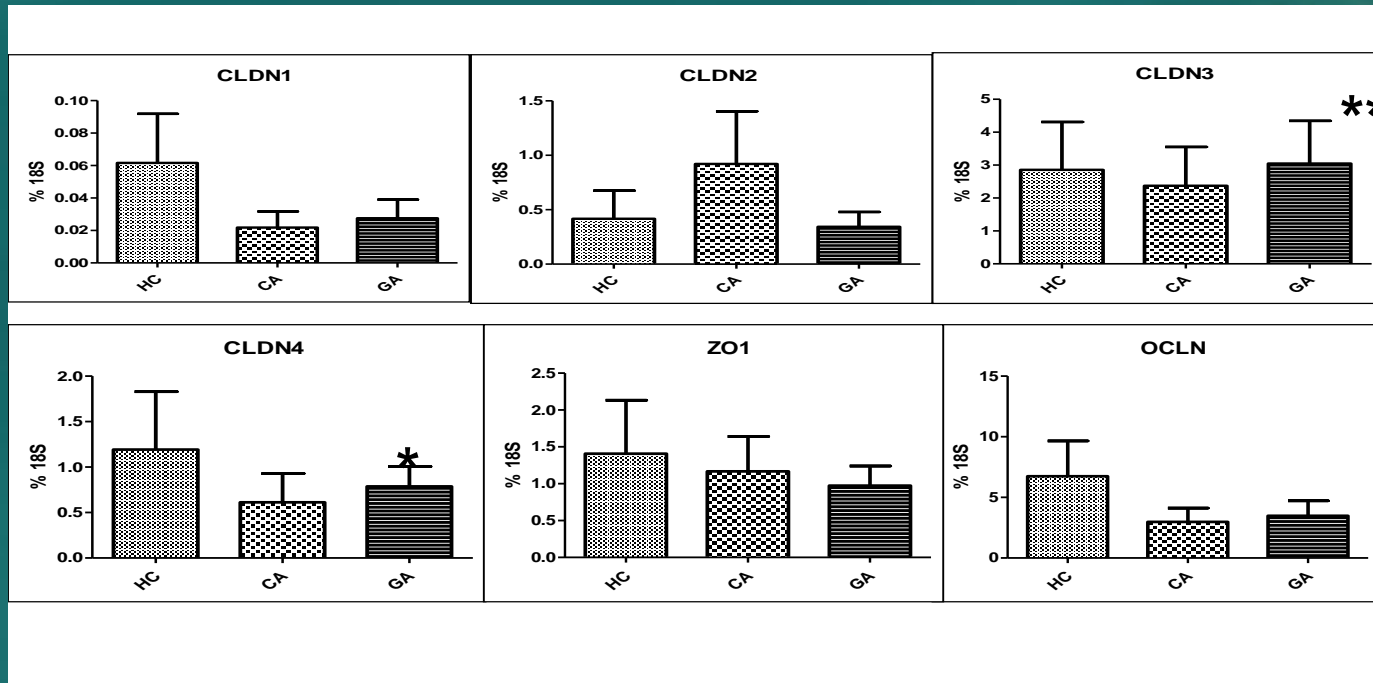
Change in Intestinal Permeability in GS and CD patients



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

Results:

Intercellular TJ expression CD



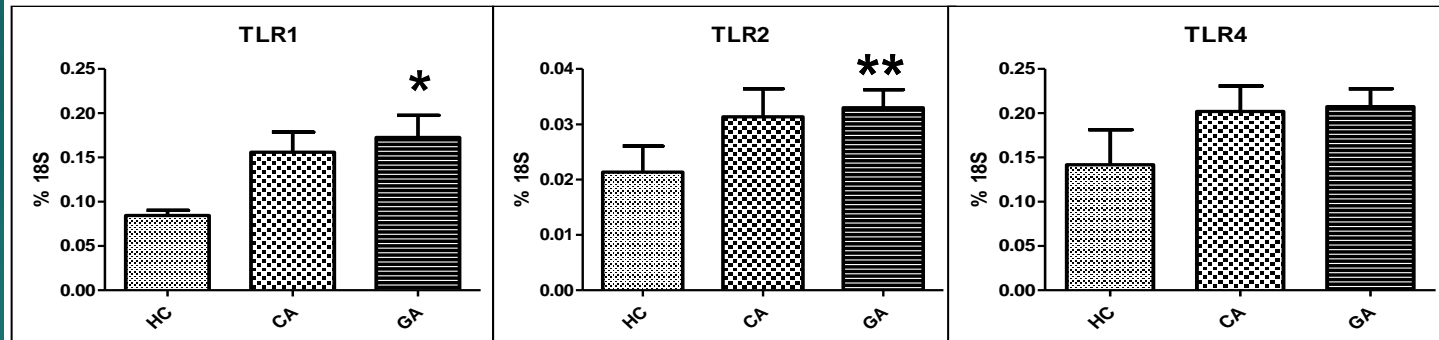
CL3 and CL4 expressions were significantly increased in GS subjects compared to CD patients ($p < 0.01$), while no changes in other TJ protein genes expression were detected. Conversely, in CD patients an over-expression of CL2 was observed that was associated to increased IP and that returned to baseline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

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

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

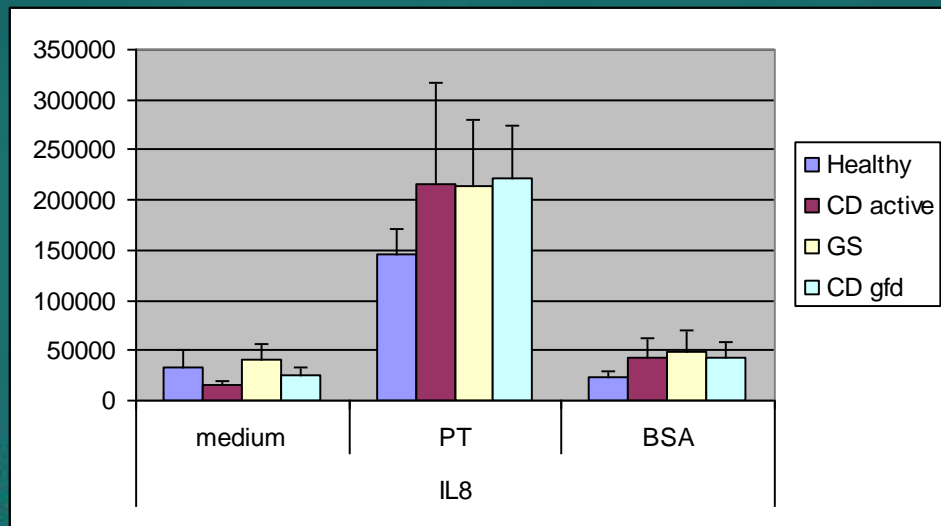
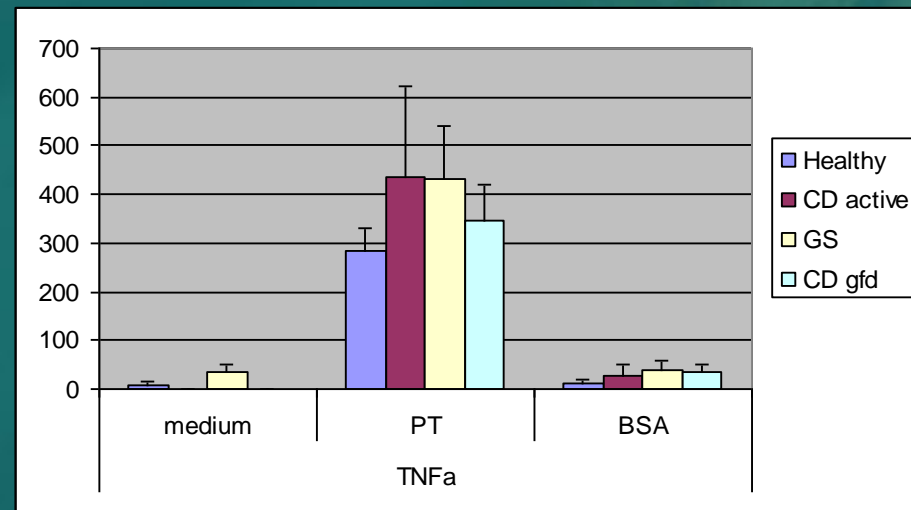
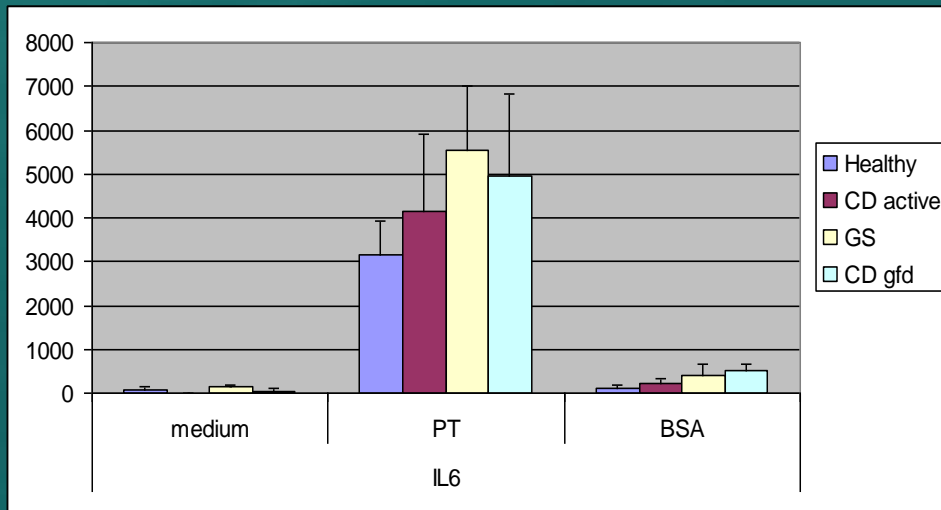
Results:

TLR signaling pathway involvement



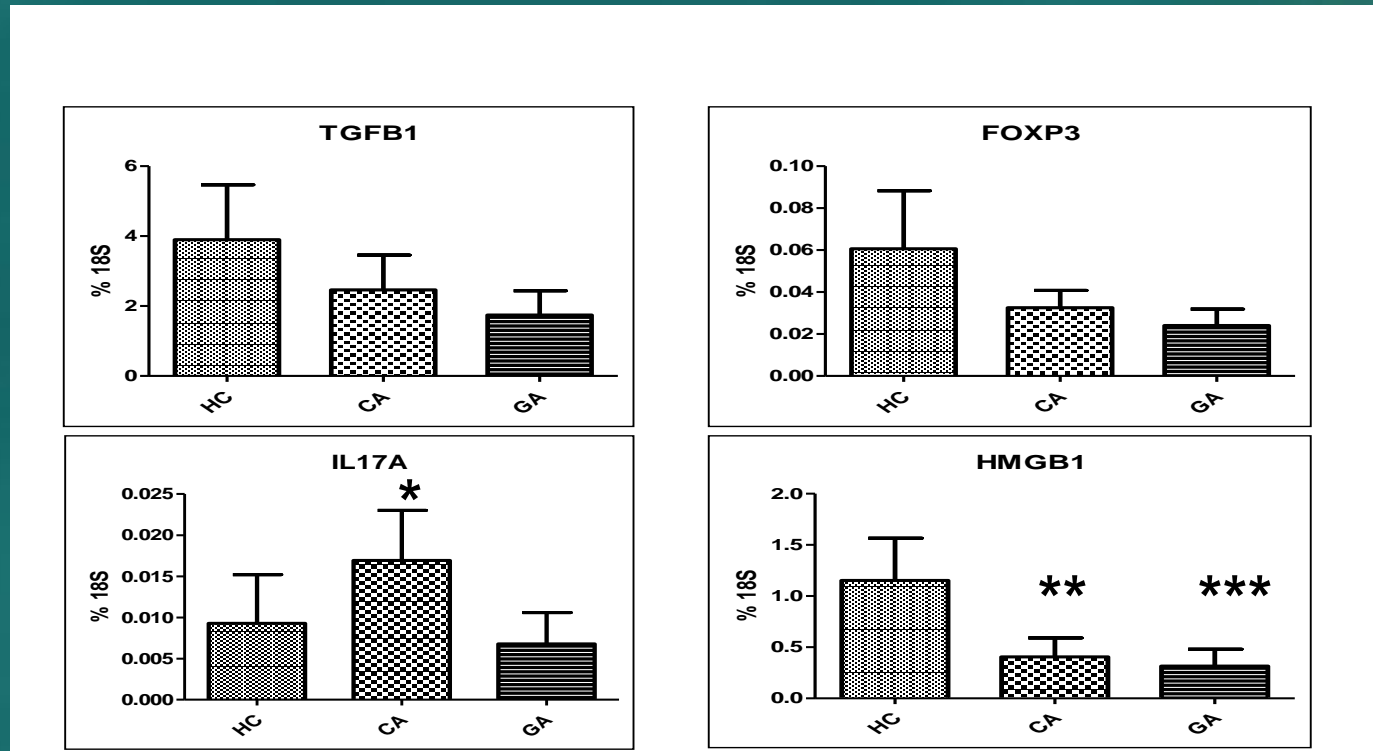
TLR1 and TLR2, but not TLR4 resulted significantly increased in GS $^{*}.$ ** ($p < 0,05$) respect to normal controls, while CD showed only a not significant increase of TLR1

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



CD pts showed a decreased expression of FOXP3 that was associated to a significantly increased of IL 17 compared to normal control and GS pts, 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

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.