

Natural History of Innate Immune Activation in Celiac Disease: triggers, pathways and, clinical implications.

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ISTITUTO: Laboratorio Europeo per lo Studio delle Malattie Indotte da Alimenti - ELFID

DURATA DEL PROGETTO: 3 anni

Outline

- Signs of epithelial stress and activation of innate immunity in CD
- What activates innate immunity in CD?
- Epithelial stress and tissue damage

Coeliac disease

T cell mediated enteropathy with an autoimmune component triggered by the ingestion of gluten and related prolamines in genetically susceptible individuals

- HLA restricted
- Th1 response by gliadin-specific CD4+ cells

However, adaptive immune response to gluten is insufficient, as suggested by:

- animal models
- potential celiac disease

Epithelial stress and activation of innate immunity

Stress as response to a threat (intrinsic or external) resulting in attempt to repair the cell damage or cell elimination (license to be killed)

Epithelial stress activates innate immunity

Signs of epithelial stress expressed by the CD epithelium

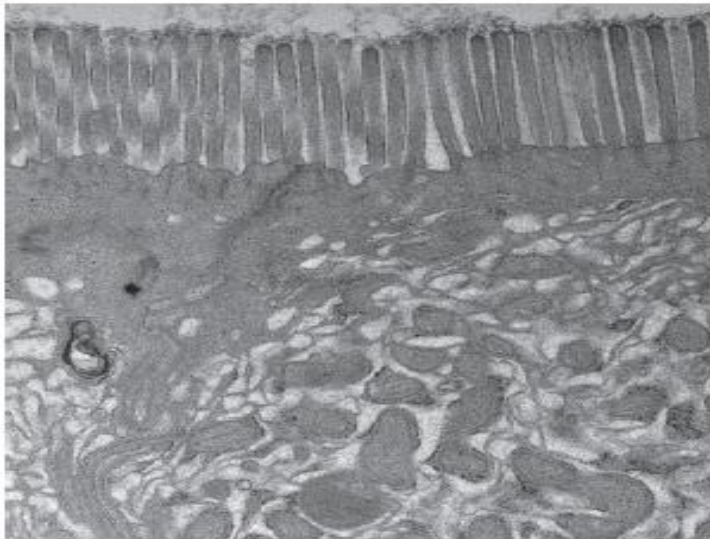
In biopsies from patients on a gluten-containing diet:

- HSP25, HSP27, HSP65, HSP70, HSP72 (Iltanen 1999, Sziksz 2010)
- MICA/B (Allegretti 2003), HLA-E (Terrazzano Immunol 2007)
- IL15 (Jabri Gastroenterology 2000, Maiuri Gastroenterology 2000, Mention Gastroenterology 2003)

Signs of epithelial stress expressed by the CD epithelium

In biopsies from patients on a gluten-containing diet alterations at EM: microvilli alterations, tight junctions

ACTIVE CD



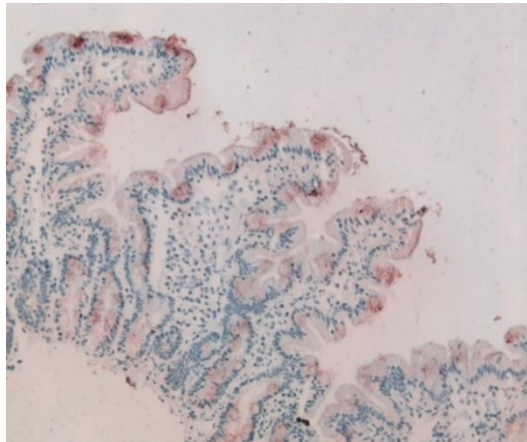
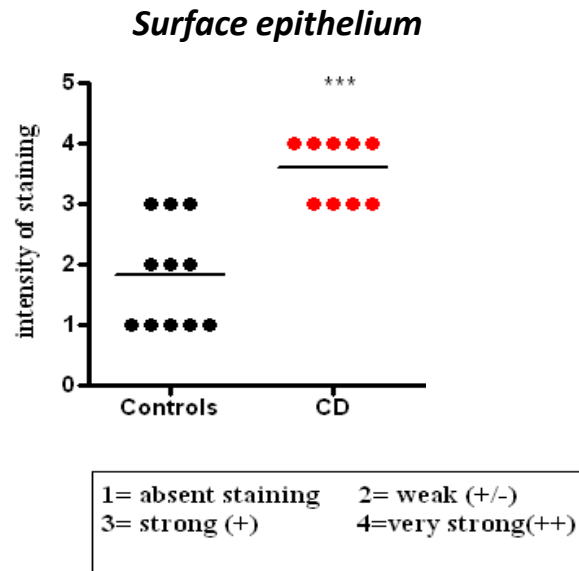
EM 3
HSP70-HSP27-IL-15+



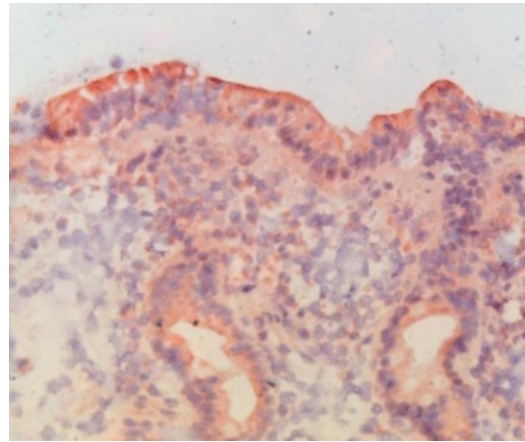
EM 4
HSP70-HSP27-IL-15-

*Setty M and Discepolo V. et al.
Gastroenterology 2015*

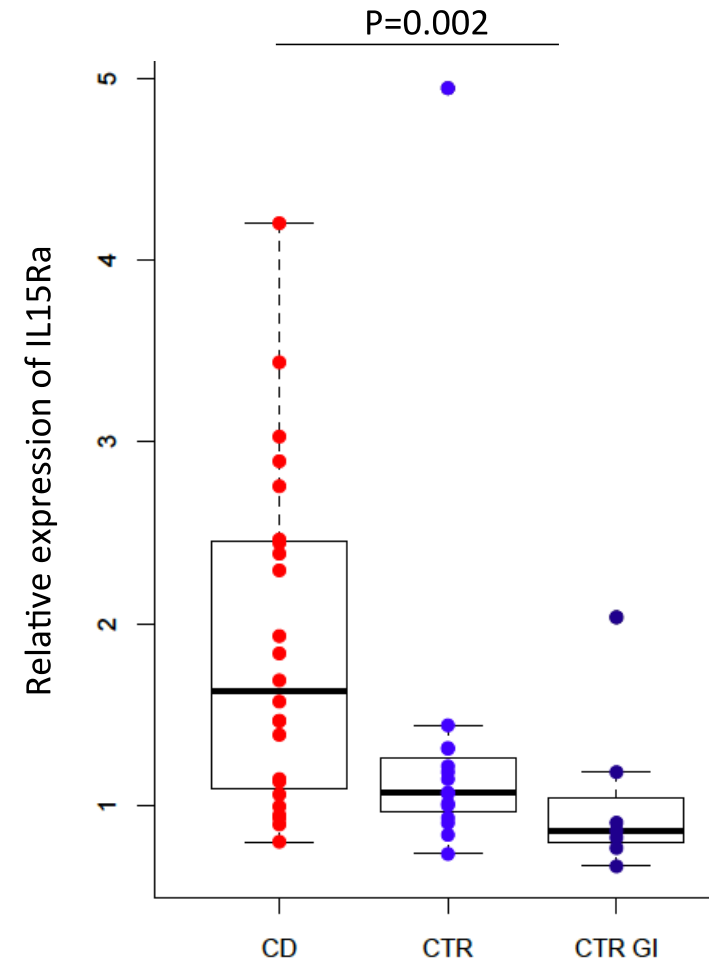
Increased IL-15 epithelial expression and increased IL-15Ra expression in biopsies of active CD patients



Control Marsh 0

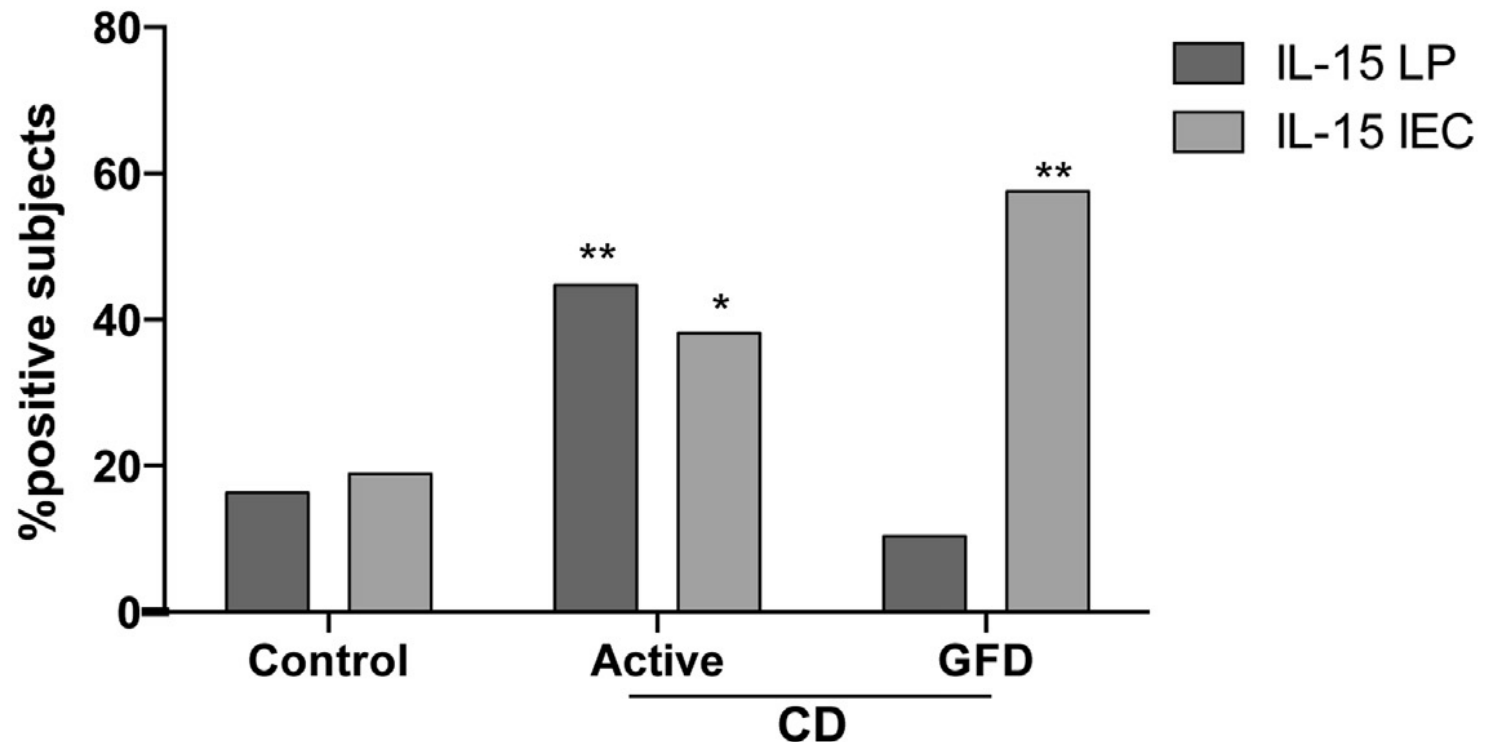


Active CD Marsh 3c



*Discepolo V et al. Unpublished
in collaboration with B Jabri*

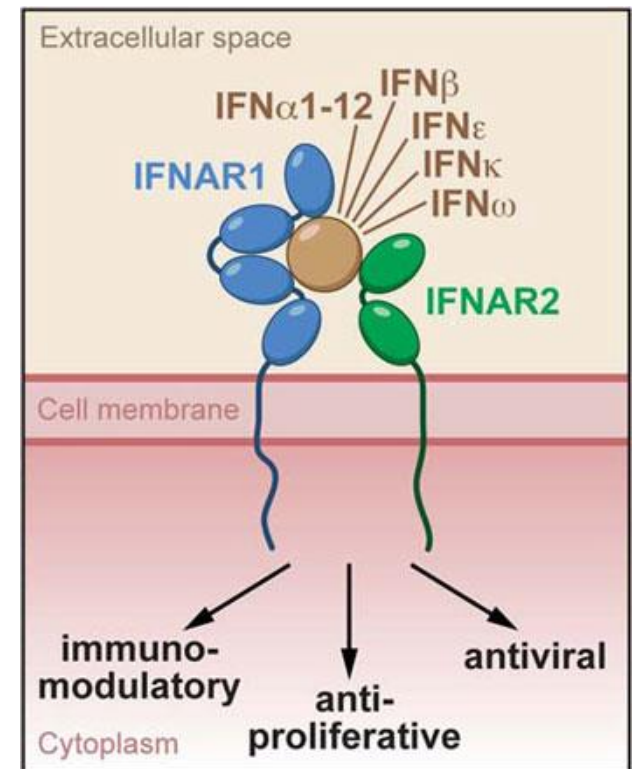
Different expression of IL15 in the epithelium and lamina propria of CD patients



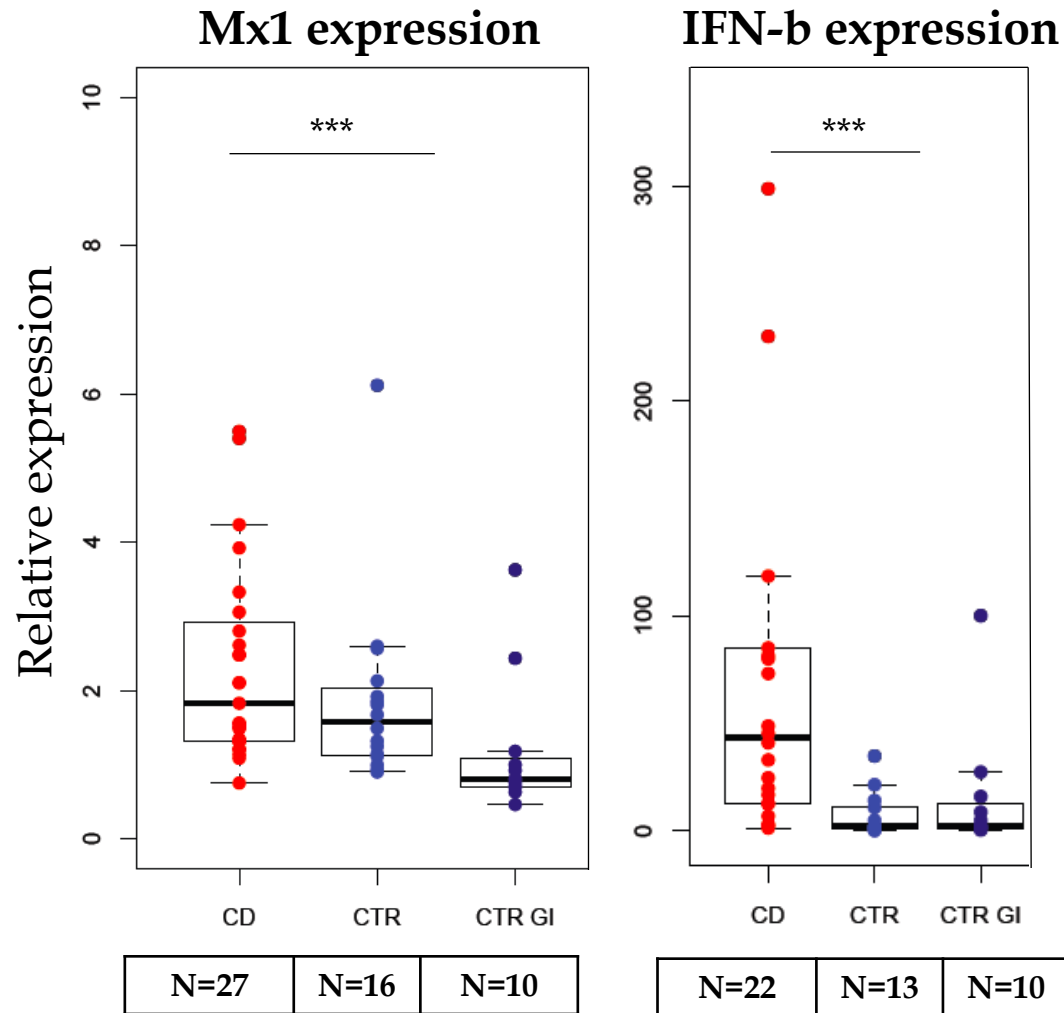
IL-15 expression in the epithelium persisted even after gluten withdrawal, while IL-15 expression in the lamina propria of CD patients decreased upon removal of gluten from the diet

Type-1 IFN, antiviral cytokines

- IFN- α IFN- β therapy elicit CD (*Lancet* 2000, *Scand J Gastroenterol* 2011)
- Increased IFN- α protein levels in active-CD biopsies (*Monteleone Gut* 2001; *Di Sabatino Gastroenterology* 2007; *Raki Mucosal Immunology* 2012)
- MxA is a protein with GTPase activity (encoded by the Mx1 gene located on the 21st chromosome) induced upon activation of the type-1 IFN signaling pathway and it is used as a proxy for IFN pathway activation

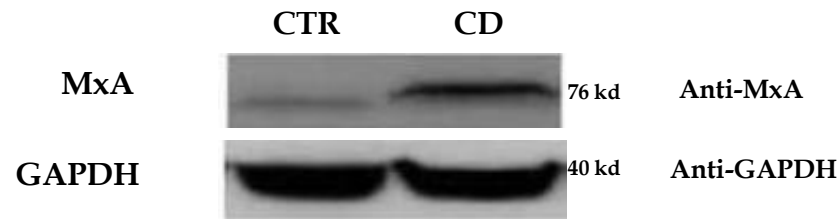


Up-regulation of type-1 IFN pathway in Active CD

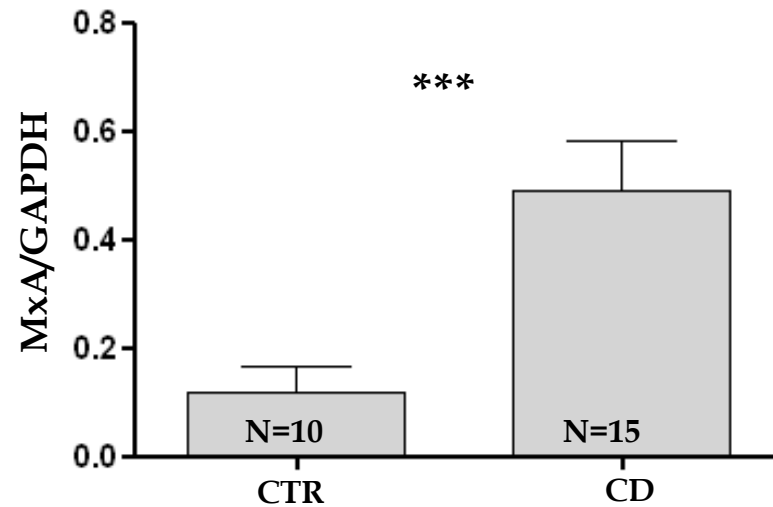


Increased levels of Mx1 and IFN-beta mRNA, as well as IFN-alpha, as assessed by rtPCR, have been found in duodenal biopsies of Active CD patients (vs CTR and CTR GI)

MxA is over-expressed in jejunal biopsies from active coeliac patients and controls



Densitometric analysis



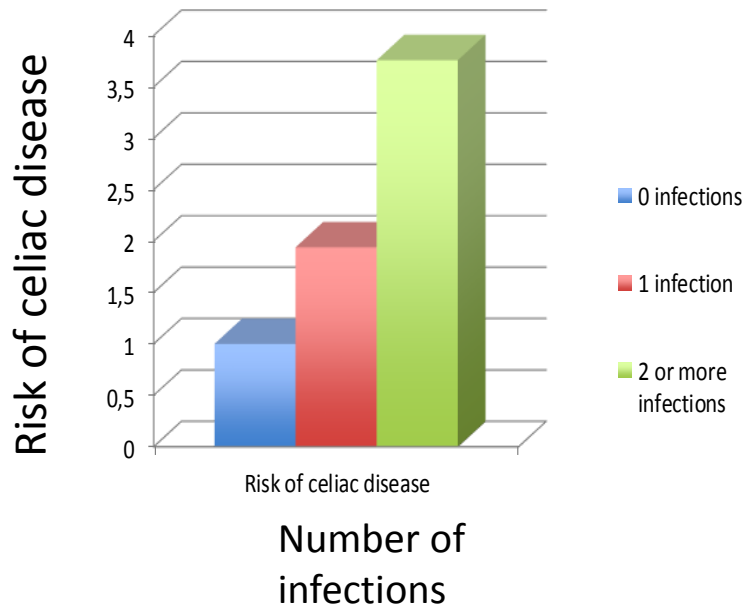
Increased levels of MxA protein (by WB) in duodenal biopsies of active CD patients

What drives epithelial stress in CD?

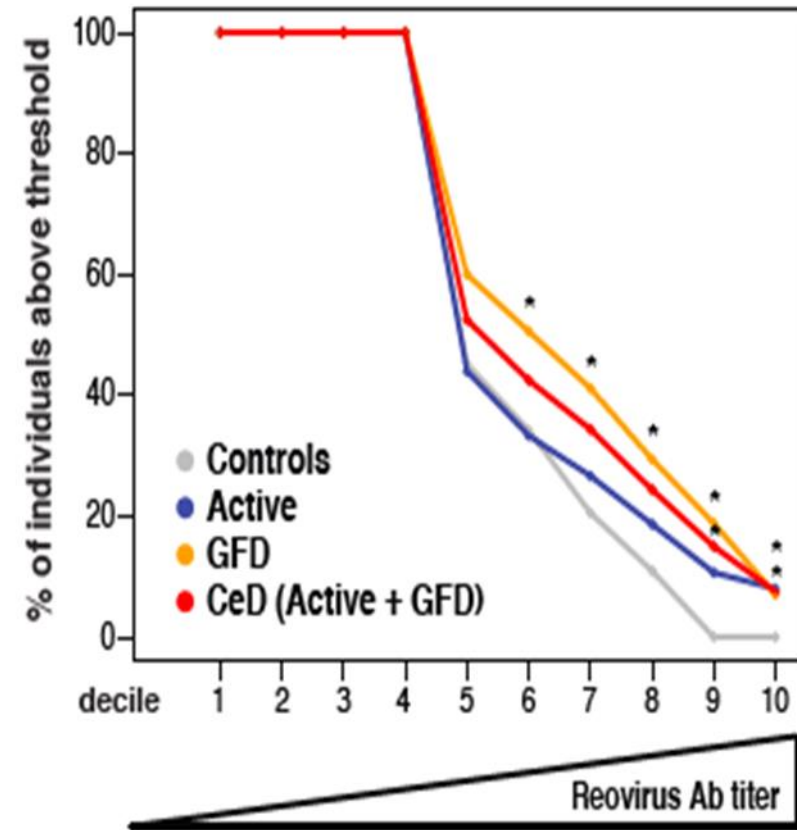
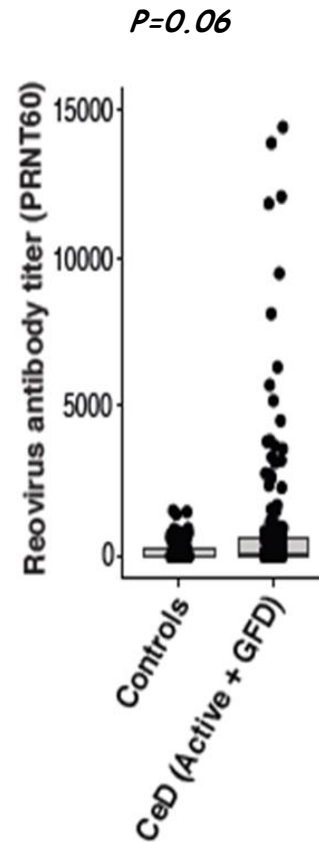
- Viral infection? Microbioma changes??
- Gliadin peptides (e.g. p31-43)?
- Constitutive alterations?

Viral infections as triggers of CD

Rotavirus infections increase the risk of celiac disease



Increased titer of anti-Reovirus antibodies in CD patients

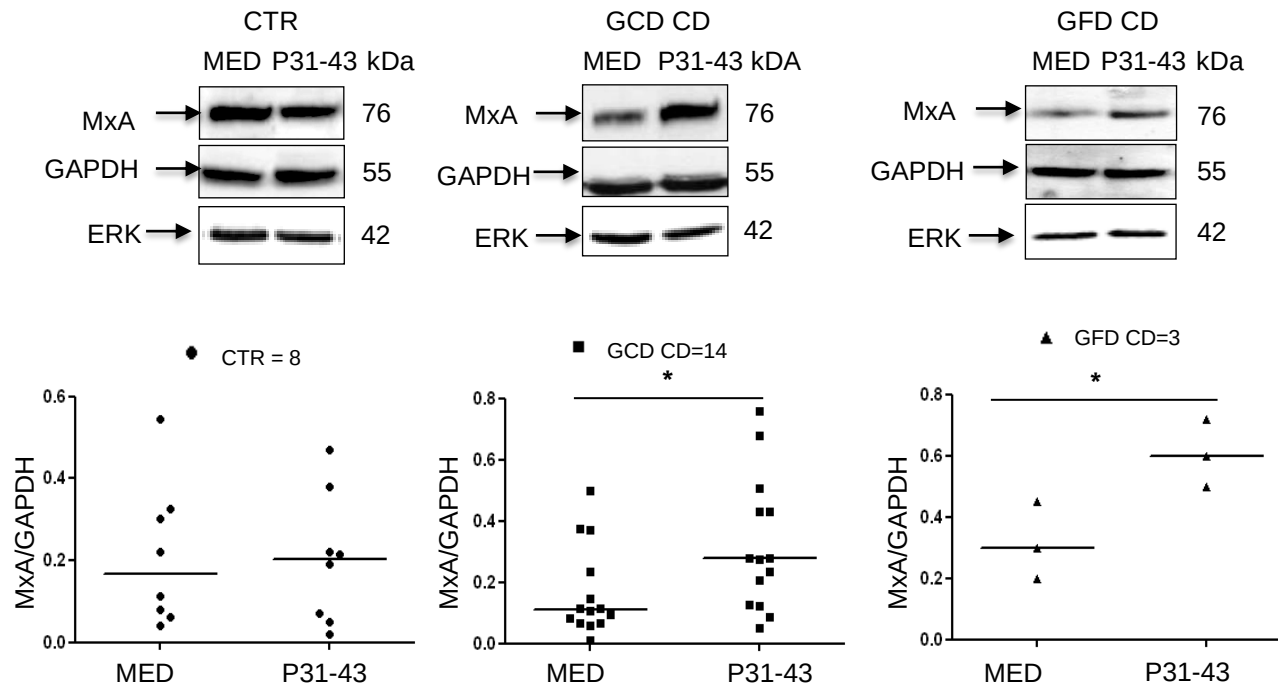


Epithelial response induced by p31-43 in CD

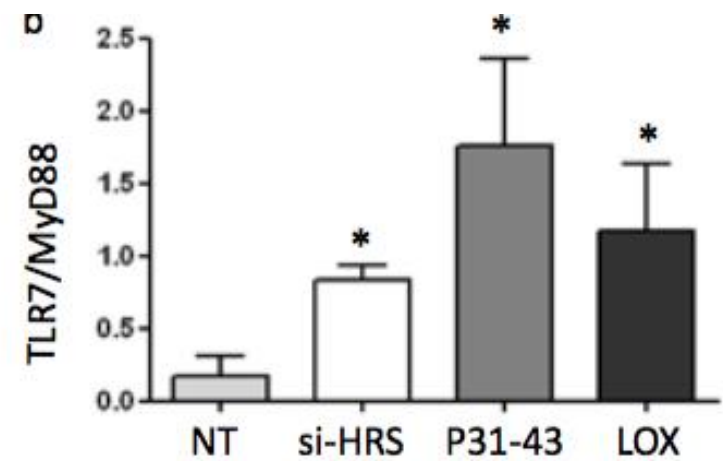
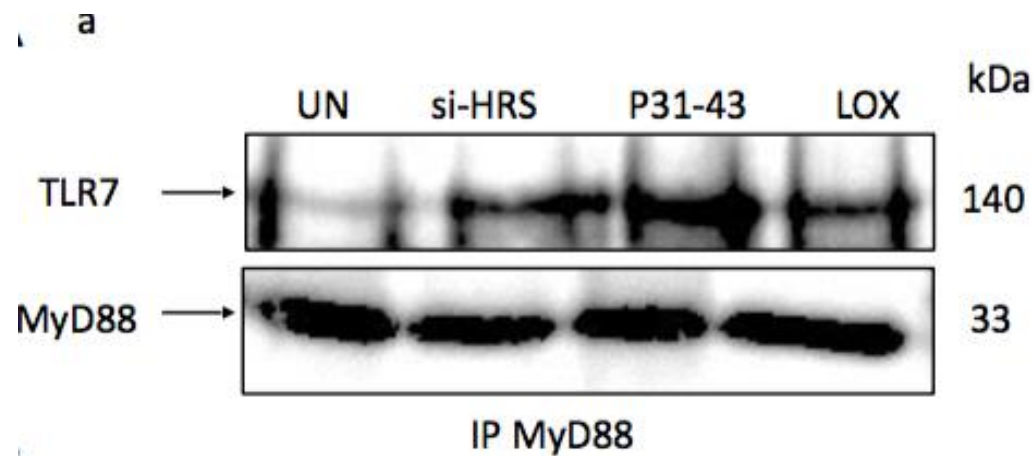
In biopsies from patients in remission cultured with gliadin peptides

- IL15 (Maiuri Lancet 2003)
- MIC A (Martin-Pagola Immunogenetics 2004, Hue Immunity 2004)
- Epithelial cell proliferation (Barone Gut 2007)

P31-43 does increase MxA expression in intestinal biopsies

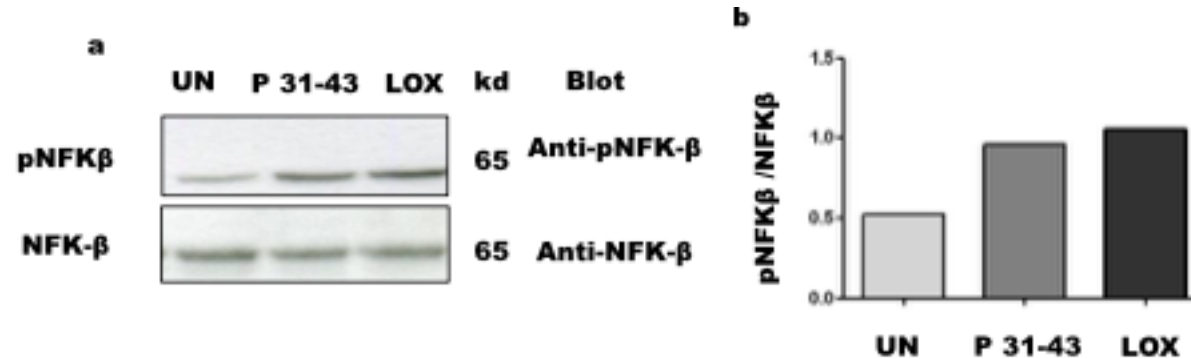


P31-43 does increase TLR7/MyD88 complex

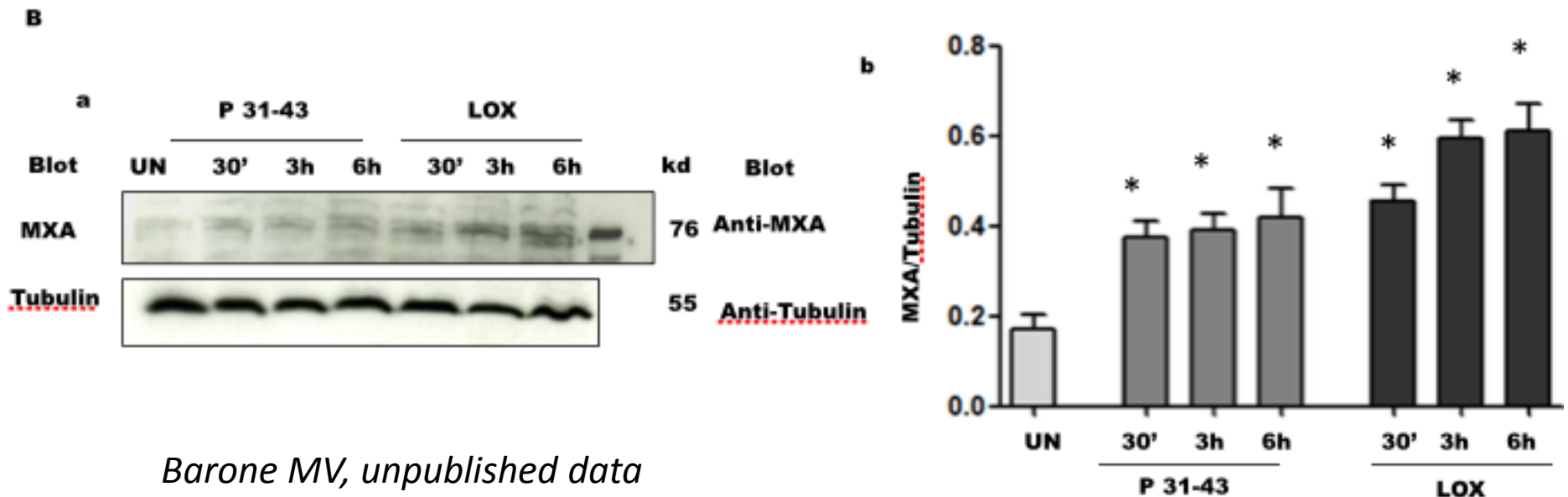


Peptide 31-43 activates NFkB and increases expression of MXA analogies with virus ligands

P31-43 like viral ligand LOX activated NFkB

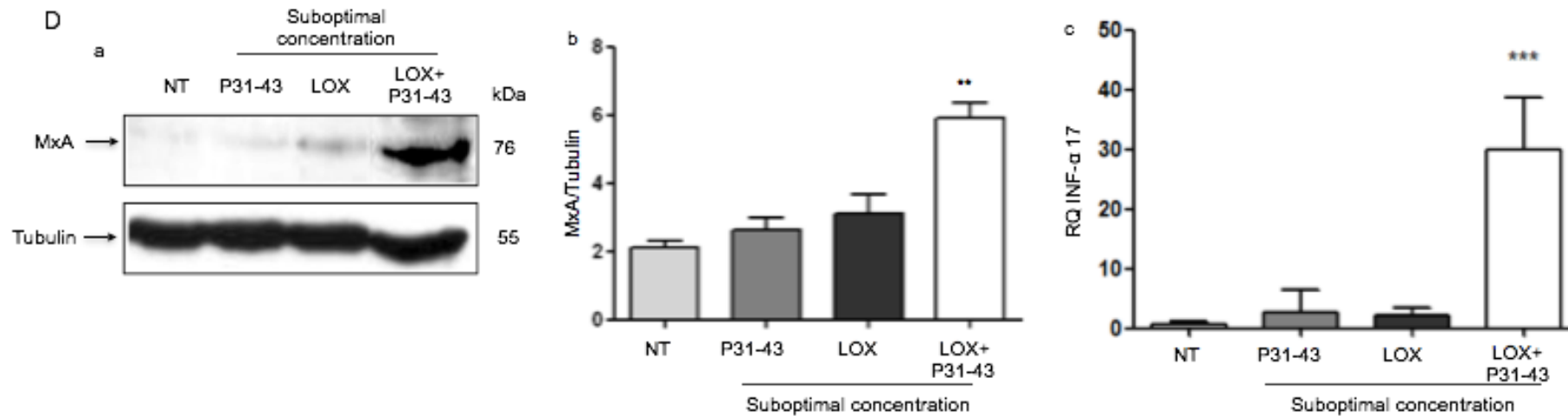


P31-43 like viral ligand LOX increased MXA levels



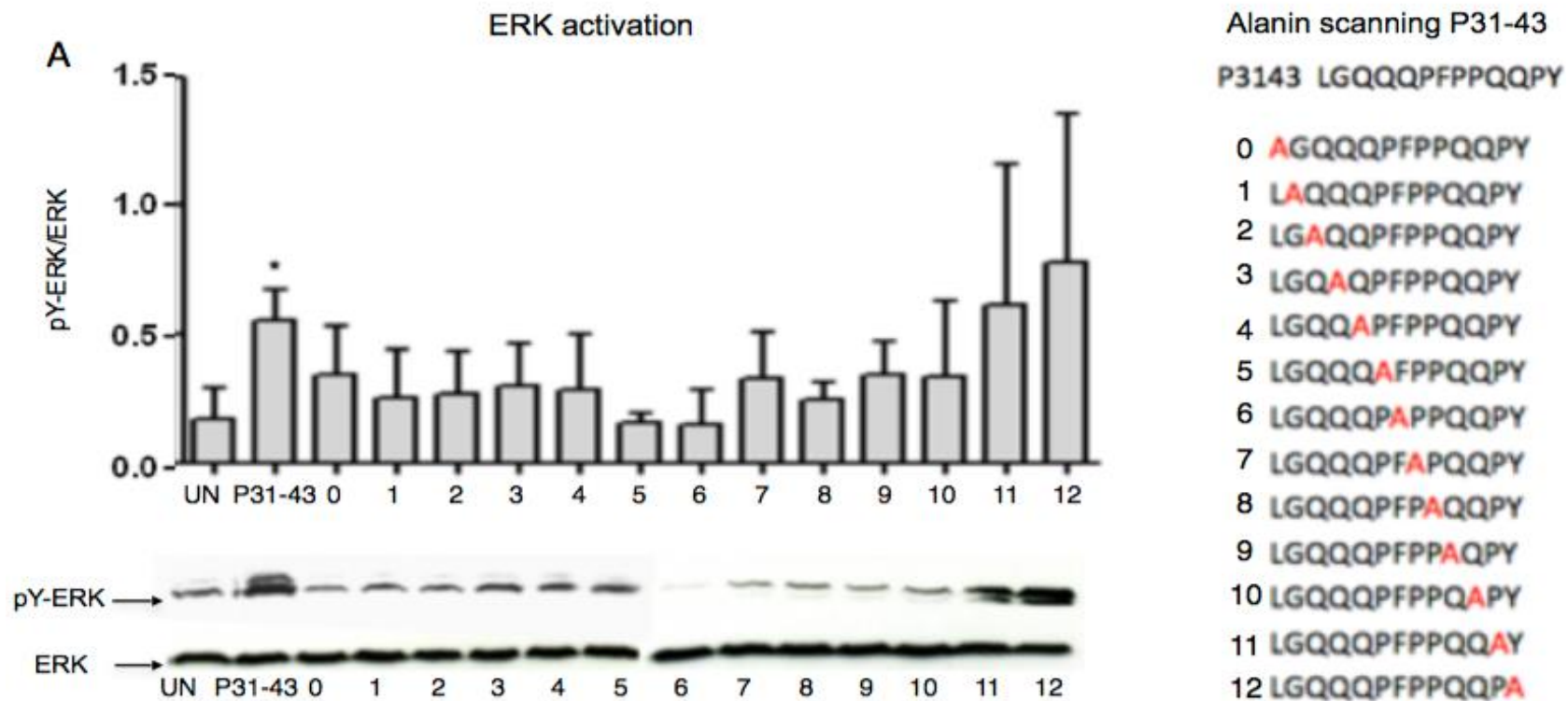
Barone MV, unpublished data

P31-43 co-operates with viral ligand LOX to induce MxA



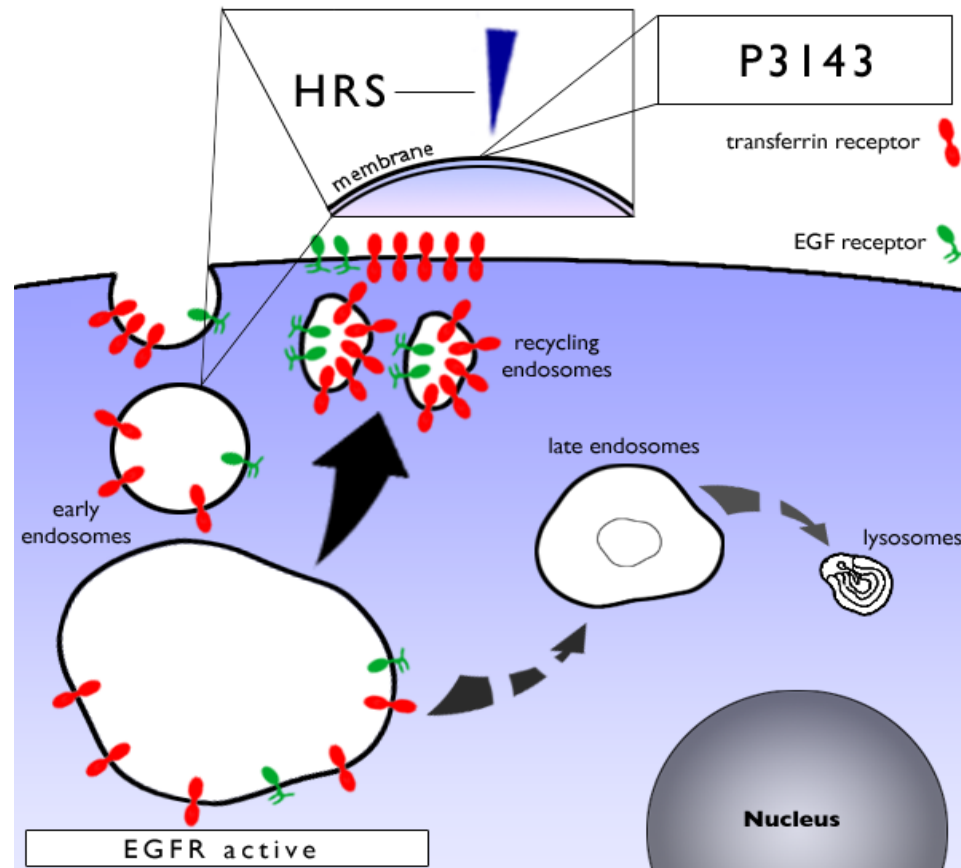
Structure of 31-43 peptide and biological effects

ALA scanning and ERK activation



How p31-43 may cause
epithelial stress?

P31-43 interferes with the endocytic pathway

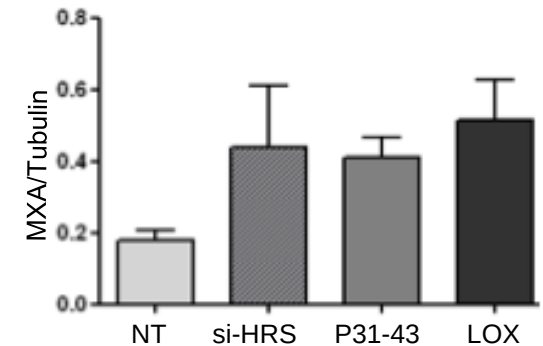
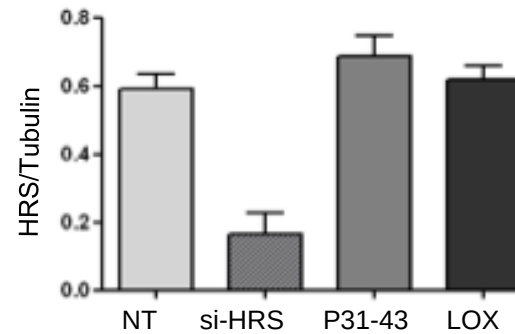
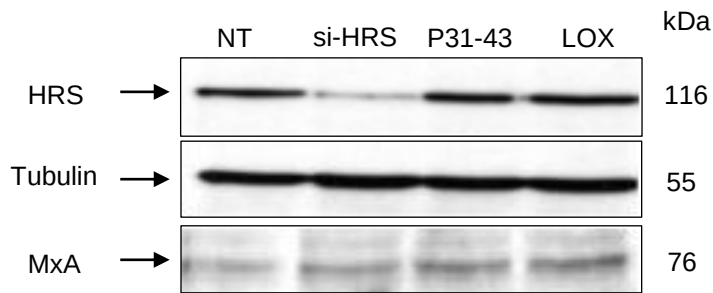


EGFR active

↓

Cytoskeleton modifications
Permeability
Motility
Proliferation
Innate immunity (?)

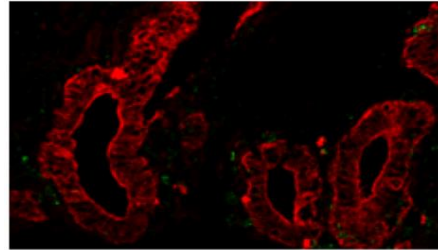
HRS silencing is per se able to induce activation of the type 1 interferon pathway



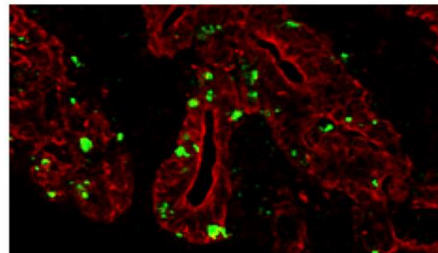
Why epithelial cells from coeliac
patients are more susceptible to
p31-43?

Increased proliferation and ERK phosphorylation in enterocytes of CD patients on GFD: a constitutive defect?

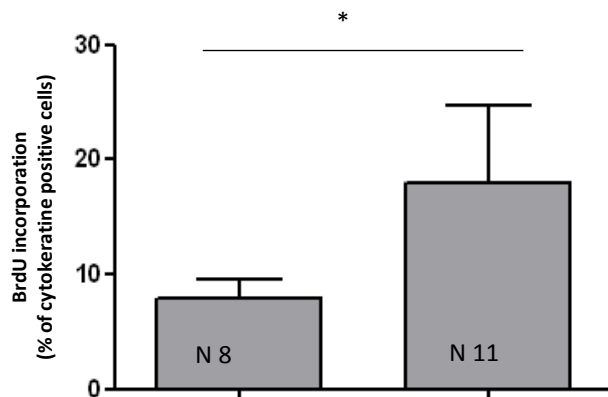
Increased proliferation



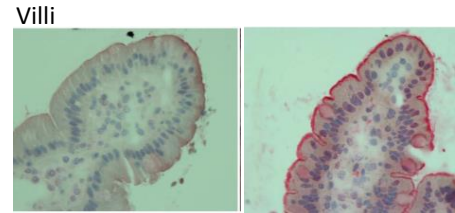
Control



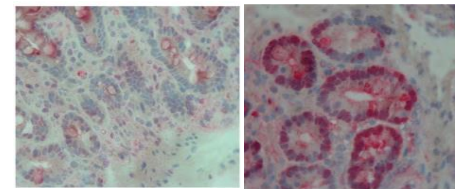
GFD CD



Increased ERK phosphorylation

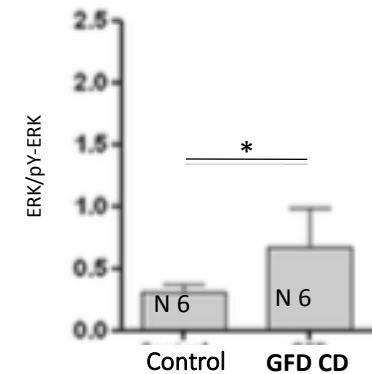
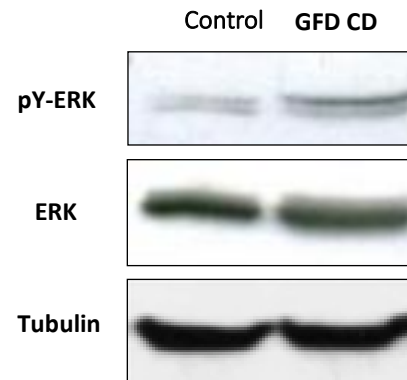
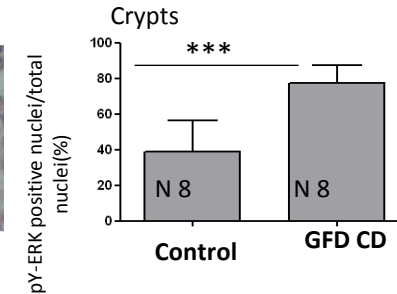
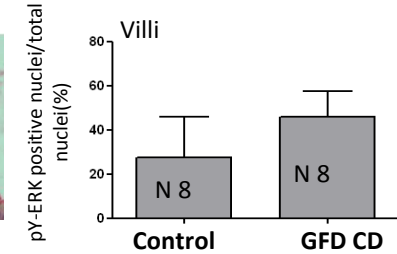


Crypts



Control

GFD CD

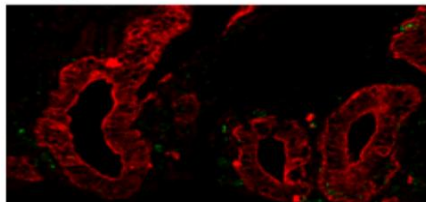


The epithelium in potential CD

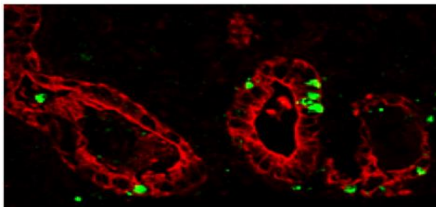
Alterations of the tight junctions (Rauhavirta et al, Transl Res 2014)

Increased proliferation and ERK phosphorylation in enterocytes (Nanayakkara M et al, PLoS One 2013)

Enterocyte proliferation

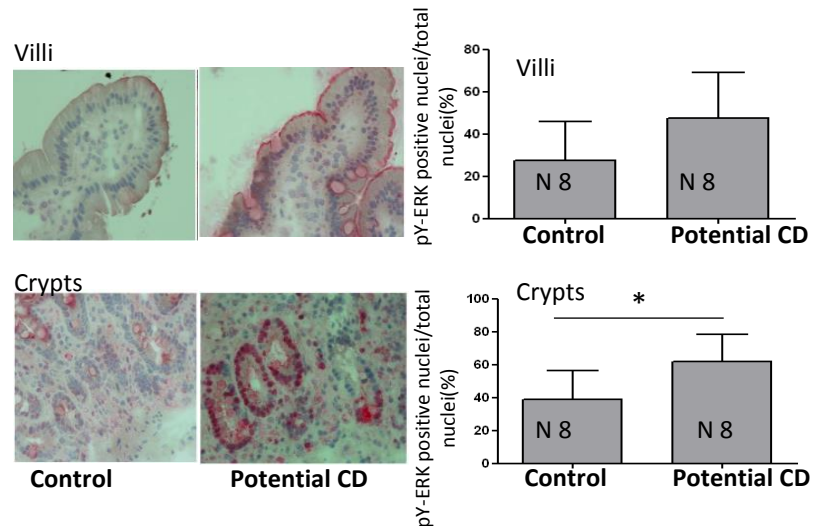


Control



Potential CD

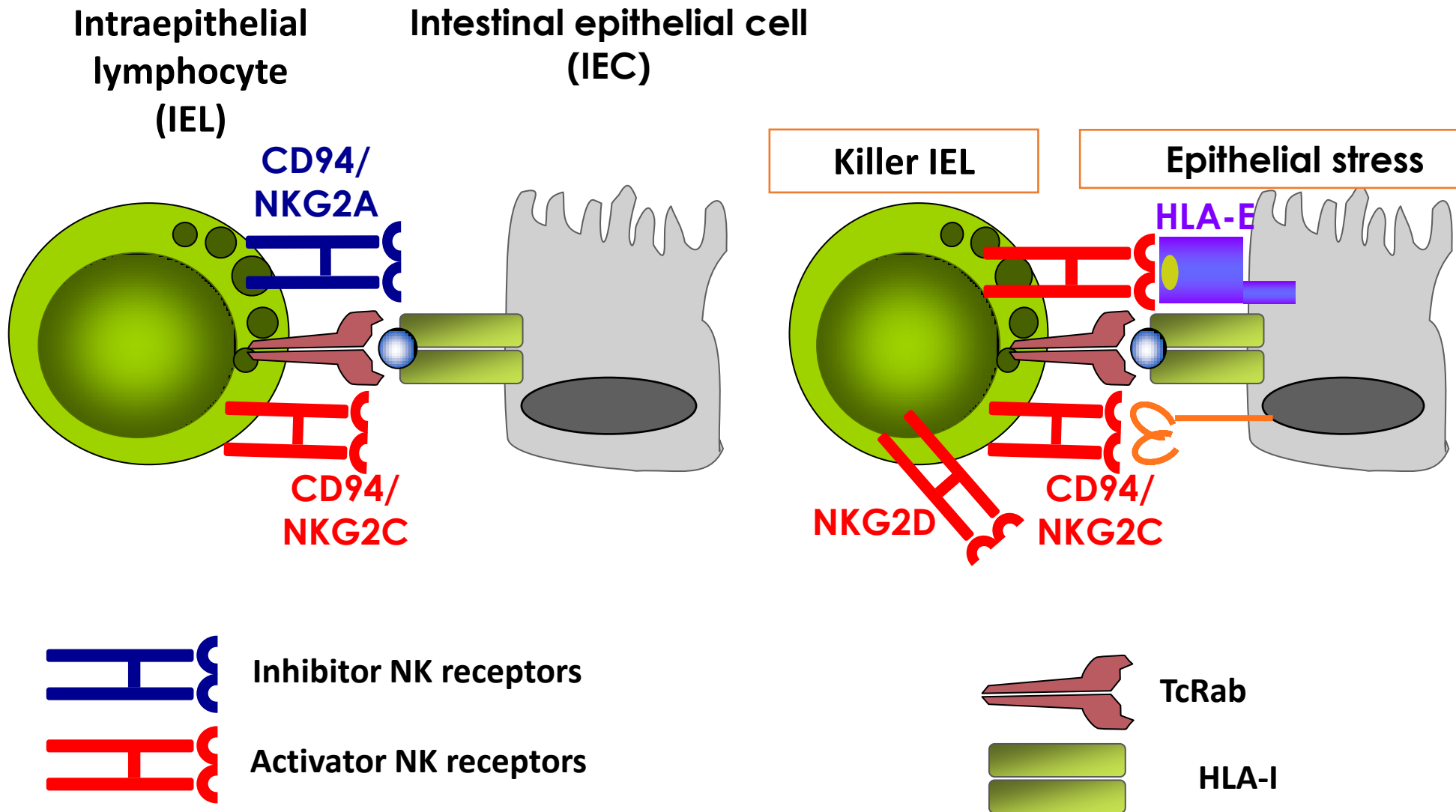
Enterocyte ERK phosphorylation



Outline

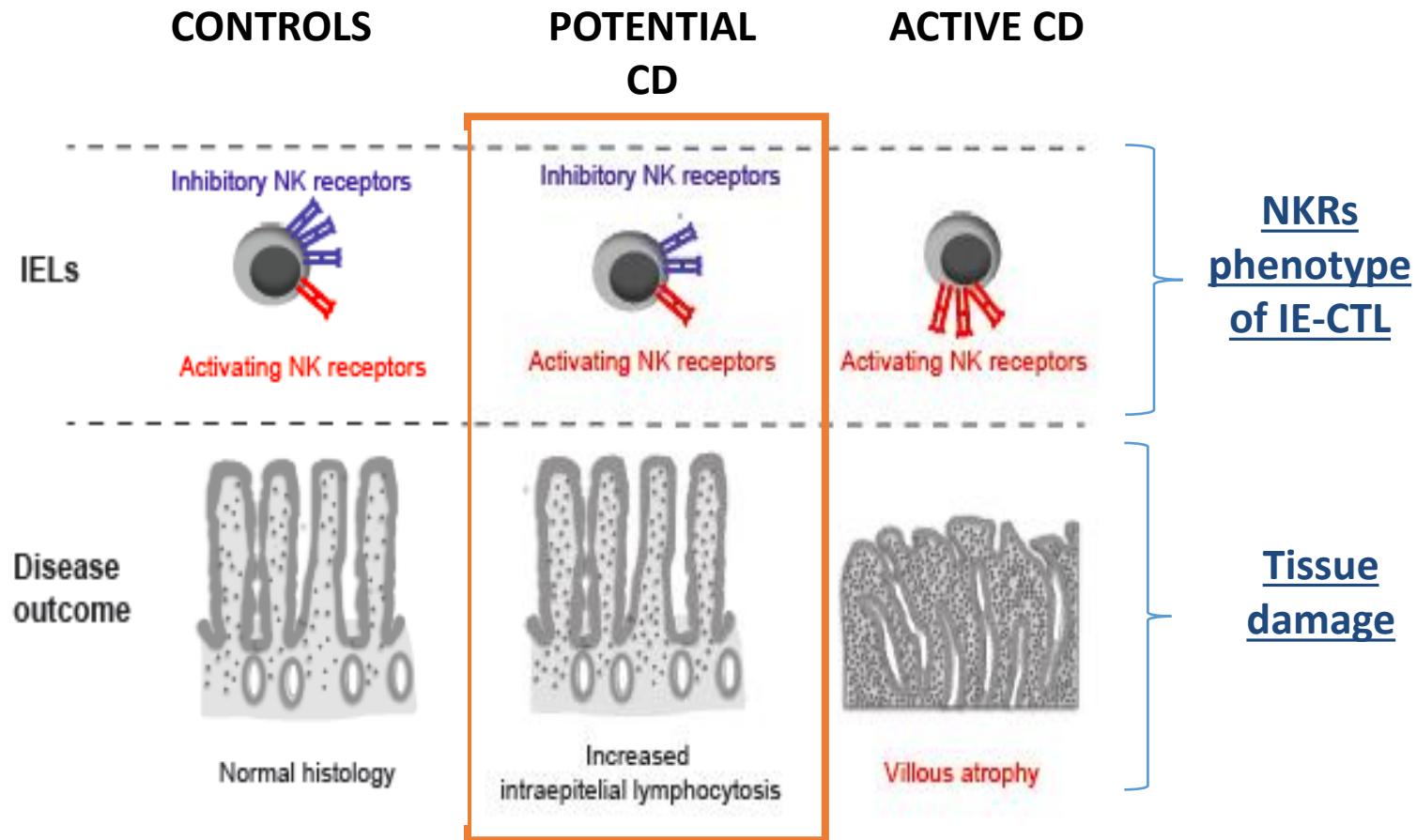
- Signs of epithelial stress
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- Epithelial stress and tissue damage

Interaction intraepithelial lymphocyte-epithelium



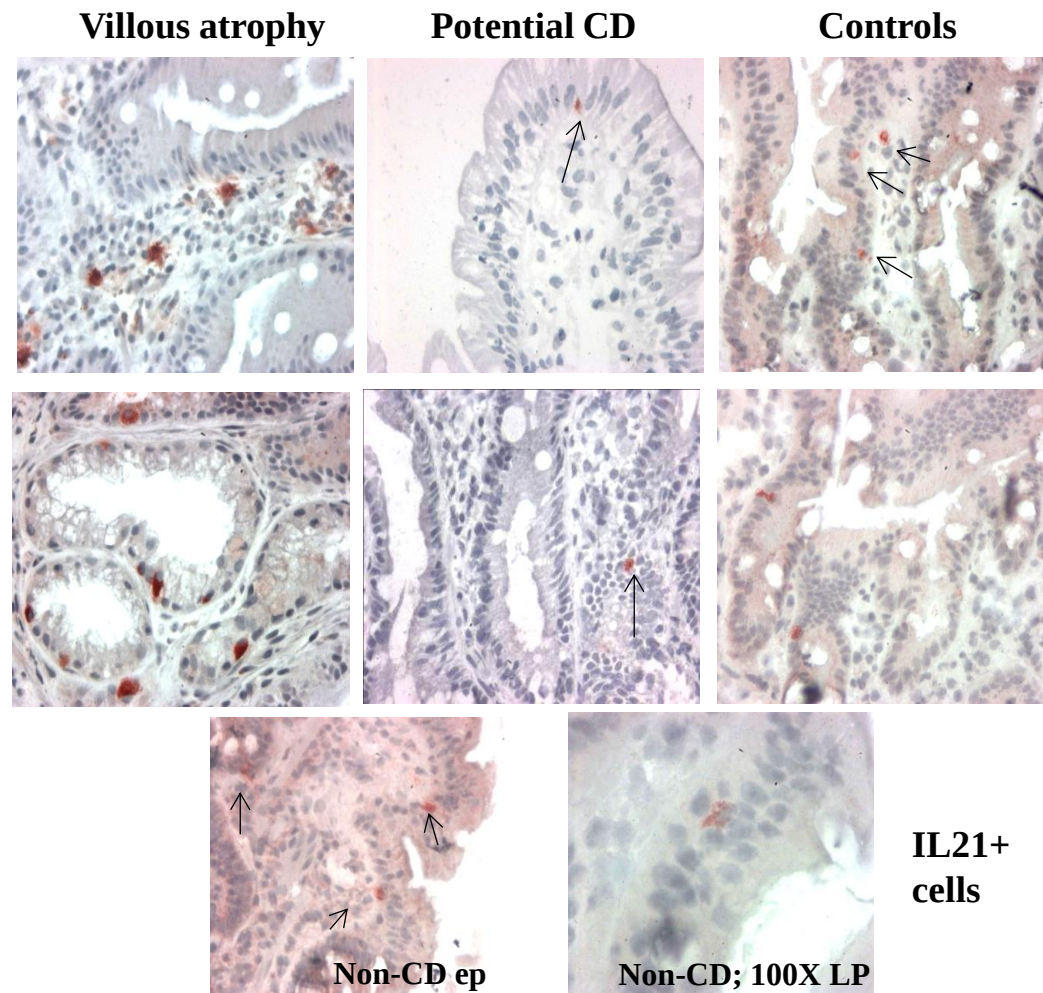
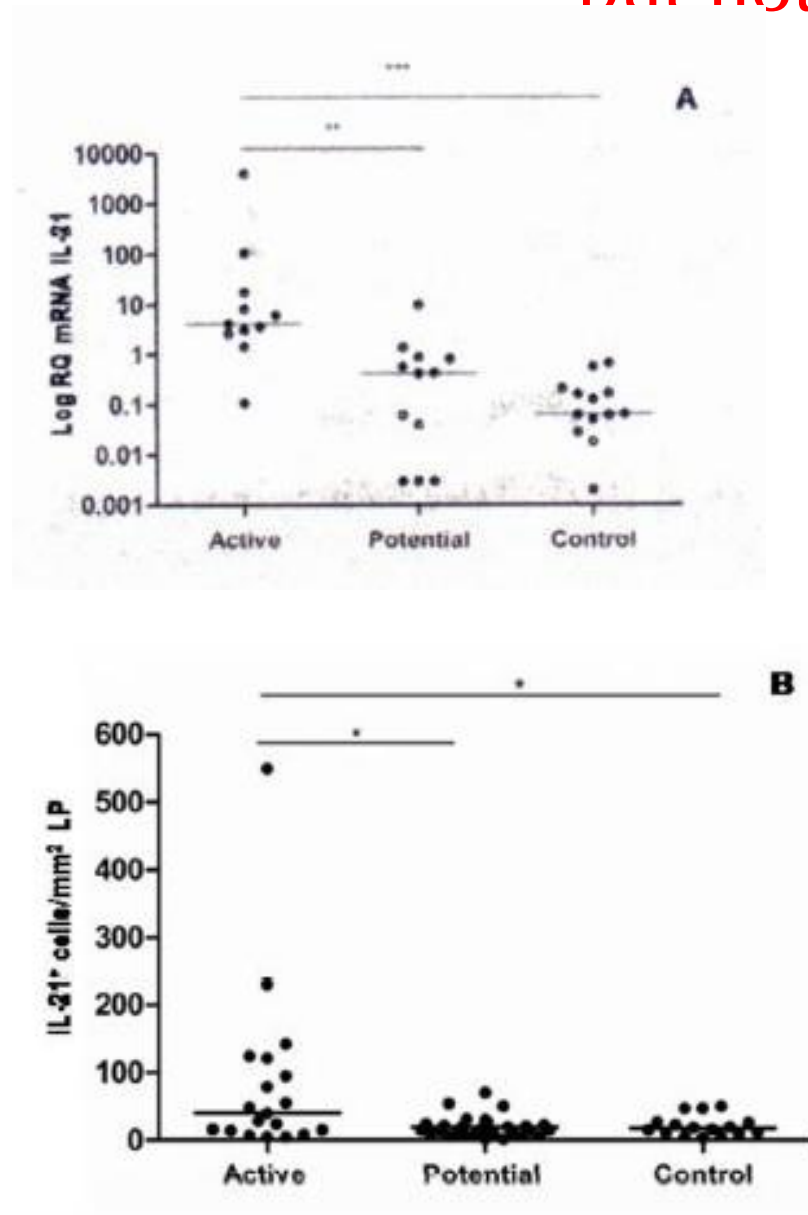
Adapted from Abadie V, Discepolo V and Jabri B, Sem Immunopath 2013

Potential CD lack villous atrophy because they have a not active NKR asset on IE-CTL



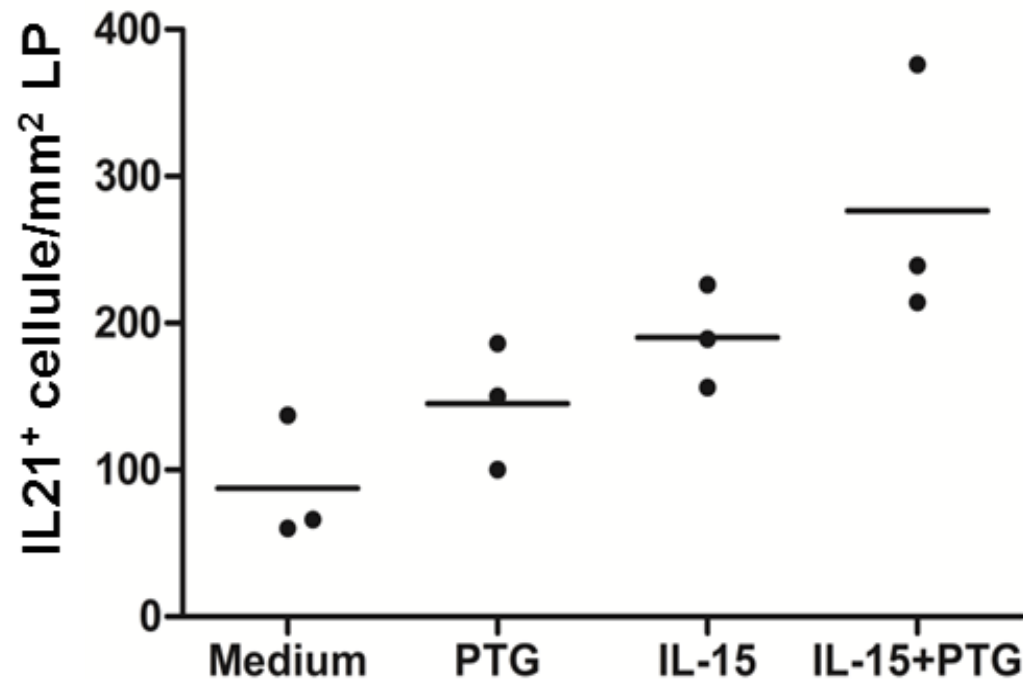
*Adopted from Setty M and Discepolo V
Gastroenterology 2016*

Up-regulation of IL-21 in Active CD, but not in potential CD



Up-regulation of IL-21 upon IL-15 stimulation

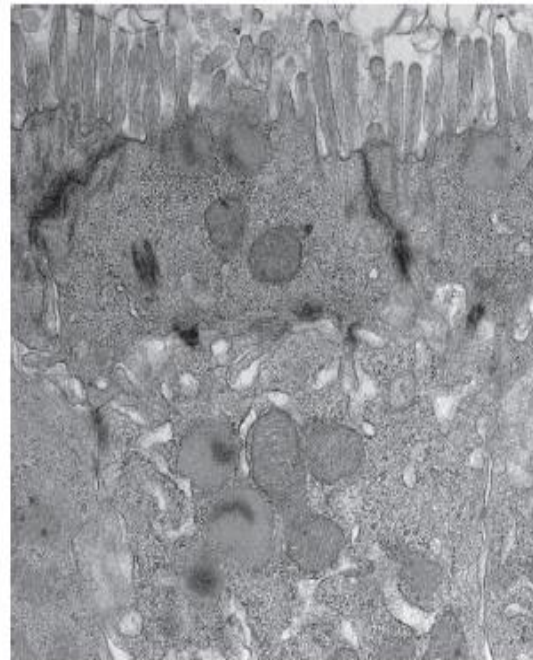
IL-15 is able to induce an up-regulation of IL-21 in the lamina propria of in vitro cultured biopsies from potential CD patients



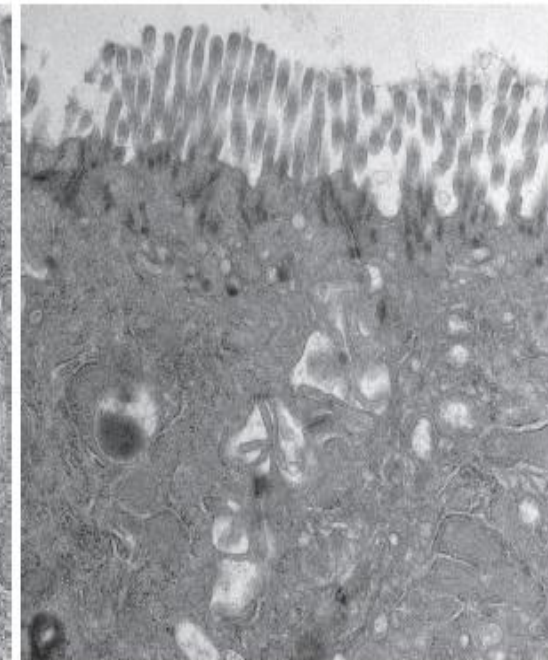
Epithelial stress response is present also in a subset of first degree relatives

Intestinal epithelial cells from FDR show alterations at EM similar to those found in active coeliac patients

AT RISK TTG-



EM 3
HSP70-HSP27-IL-15+

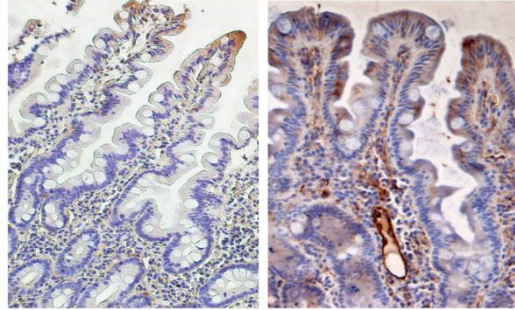


EM 4
HSP70-HSP27+IL-15-

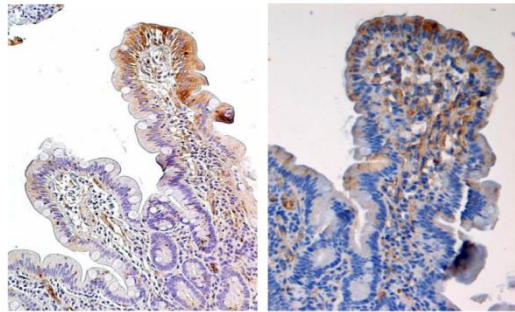
*Setty M and Discepolo V. et al.
Gastroenterology 2015*

Increased expression of epithelial stress markers in family relatives of celiac patients

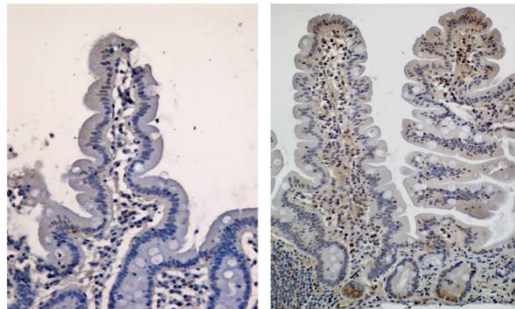
Hsp-27



Hsp-70

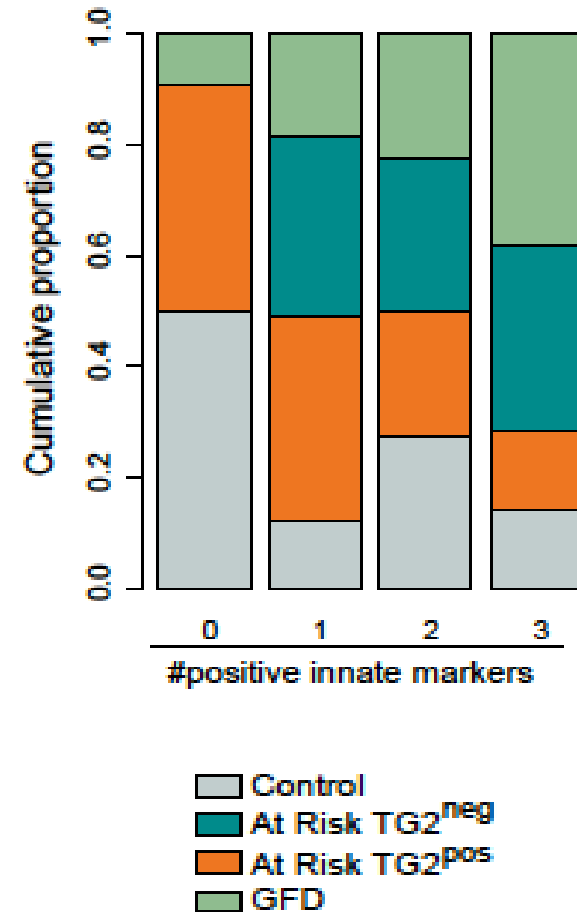


IL-15



Immunohistochemistry to evaluate Epithelial stress markers

- Heat shock proteins (HSP)
- Innate cytokine IL-15



Family relatives (At Risk TG2^{neg}) express higher number of epithelial stress markers as compared to potential CD

Other evidences suggesting alterations in first degree relatives

- 41% of FDR show increased density of gammadelta IELs (Holm Lancet 1992)
- 25% of FDR, most seronegative, have a Marsh 1 lesion (Esteve Gut 2006)
- In a subset of FDR rectal gluten challenge induces $\gamma\delta$ T IELs recruitment (Troncone Gastroenterology 1996)

Conclusions

- In celiac disease signs of epithelial stress are present
- This is result of a constitutive alterations and/or gliadin effect and/or other environmental factors (virus?)
- The stress response renders epithelial cells susceptible to killing
- The stress response is not limited to patients, but present also in a subset of FDR, suggesting a genetic basis. Prospective studies in family members will help to understand which is the trigger

Acknowledgments

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Giuliana Lania
Merlin Nanayakkara

Carmen Gianfrani
Renata Auricchio
Melissa Borrelli



Publications

1. Setty M, Discepolo V, Abadie V, Kamhawi S, Mayassi T, Kent A, Ciszewski C, Maglio M, Kistner E, Bhagat G, Semrad C, Kupfer SS, Green PH, Guandalini S, Troncone R, Murray JA, Turner JR, Jabri B. Distinct and synergistic contributions of epithelial stress and adaptive immunity to functions of intraepithelial killer cells and active celiac disease. *Gastroenterology* 2015;149:681-91.
2. Tosco A, Maglio M, Paparo F, Greco L, Troncone R, Auricchio R. Discriminant score for celiac disease based on immunohistochemical analysis of duodenal biopsies. *J Pediatr Gastroenterol Nutr.* 2015;60:621-5.
3. Borrelli M, Gianfrani C, Lania G, Aitoro R, Ferrara K, Nanayakkara M, Ponticelli D, Zanzi D, Discepolo V, Vitale S, Barone MV, Troncone R, Auricchio R, Maglio M. In the Intestinal Mucosa of Children With Potential Celiac Disease IL-21 and IL-17A are Less Expressed than in the Active Disease. *Am J Gastroenterol.* 2016;111:134-44.
4. Paolella G, Lepretti M, Barone MV, Nanayakkara M, Di Zenzo M, Sblattero D, Auricchio S, Esposito C, Caputo I. Celiac anti-type 2 transglutaminase antibodies induce differential effects in fibroblasts from celiac disease patients and from healthy subjects. *Amino Acids.* 2017;49:541-550.

Publications

1. Camarca A, Auricchio R, Picascia S, Fierro O, Maglio M, Miele E, Malamisura B, Greco L, Troncone R, Gianfrani C. Gliadin-reactive T cells in Italian children from preventCD cohort at high risk of celiac disease. *Pediatr Allergy Immunol* 2017;28:362-369.
2. Nanayakkara M, Lania G, Maglio M, Auricchio R, De Musis C, Discepolo V, Miele E, Jabri B, Troncone R, Auricchio S, Barone Mv. A-gliadin undigested peptide activates the IFN-alpha pathway in enterocytes by interfering with endocytic trafficking [Submitted]
3. Lania G, Nanayakkara M, Maglio M, Auricchio R, De Matteis A, Rizzo R, Luini A, Discepolo V, Troncone R, Auricchio S, Barone MV. Constitutive alterations of vesicular trafficking predispose to the innate immune response to gliadin in Celiac Disease. [Submitted]