Wheat Amylase Trypsin Inhibitors as Triggers of Innate Immunity

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Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL

JOHANNES GUTENBERG UNIVERSITÄT MAINZ


DON'T FORGET, YOU ARE WHAT YOU EAT.
I NEED TO EAT A SKINNY PERSON.

Food intolerances

1. Lactose or fructose intolerance
2. “Histamine intolerance”
3. Food allergy
4. Celiac disease (gluten: wheat, barley, rye)

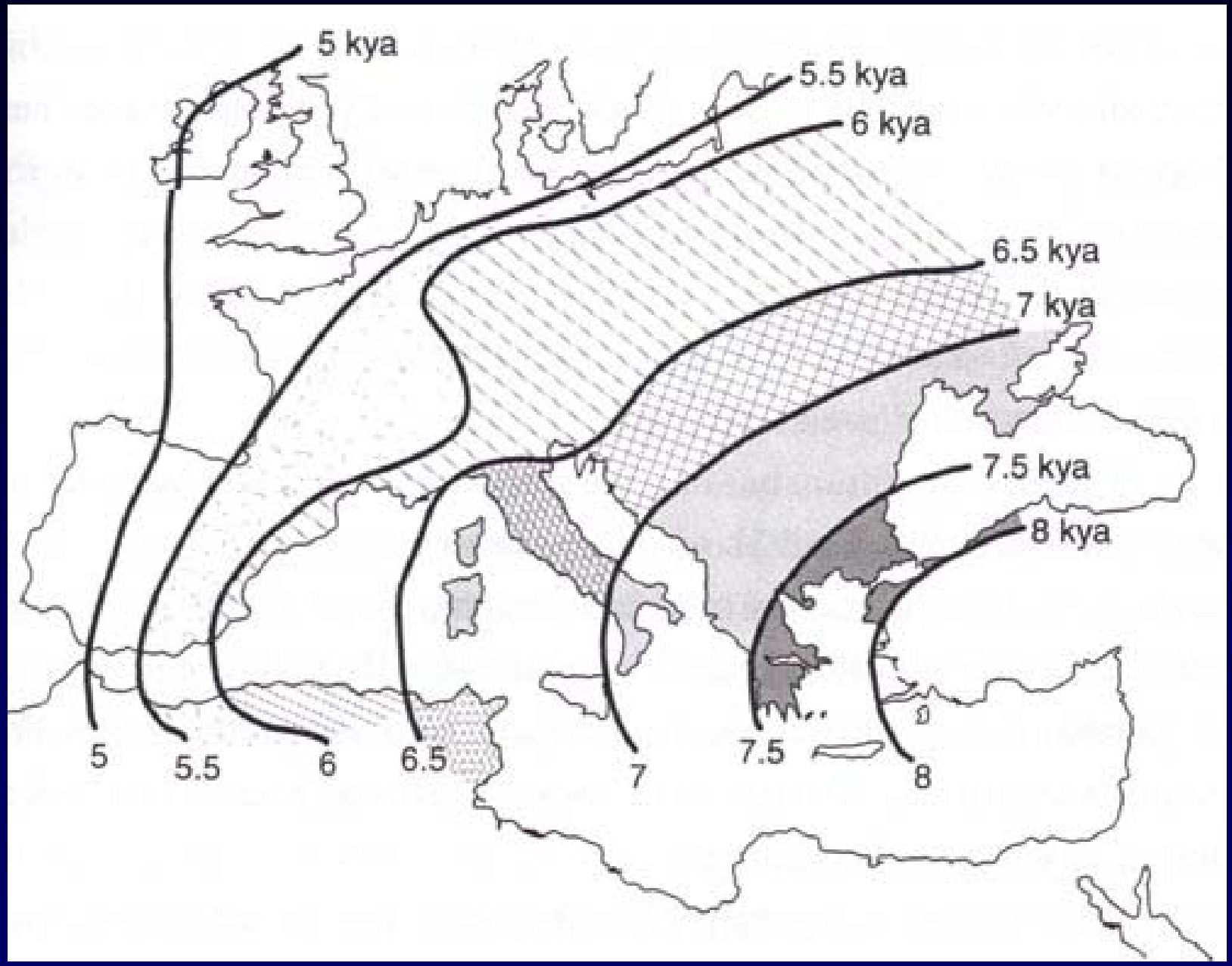
Recent – very common

5. Non-celiac “gluten” sensitivity
6. FODMAP intolerance

Frequently associated

6. Irritable bowel syndrome
7. Pathological intestinal microbiota


HELP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Mesopotamia

Egypt

Moderner Massenanbau

Opening Pandora's Bread Box

Hallmarks of celiac disease

- Dietary gluten from wheat, barley, or rye as trigger of adaptive (T cell) immunity
- Genetic Predisposition (HLA-DQ2 or -DQ8)
- IgA autoantibodies to tissue transglutaminase
- A wheat component that drives innate immunity

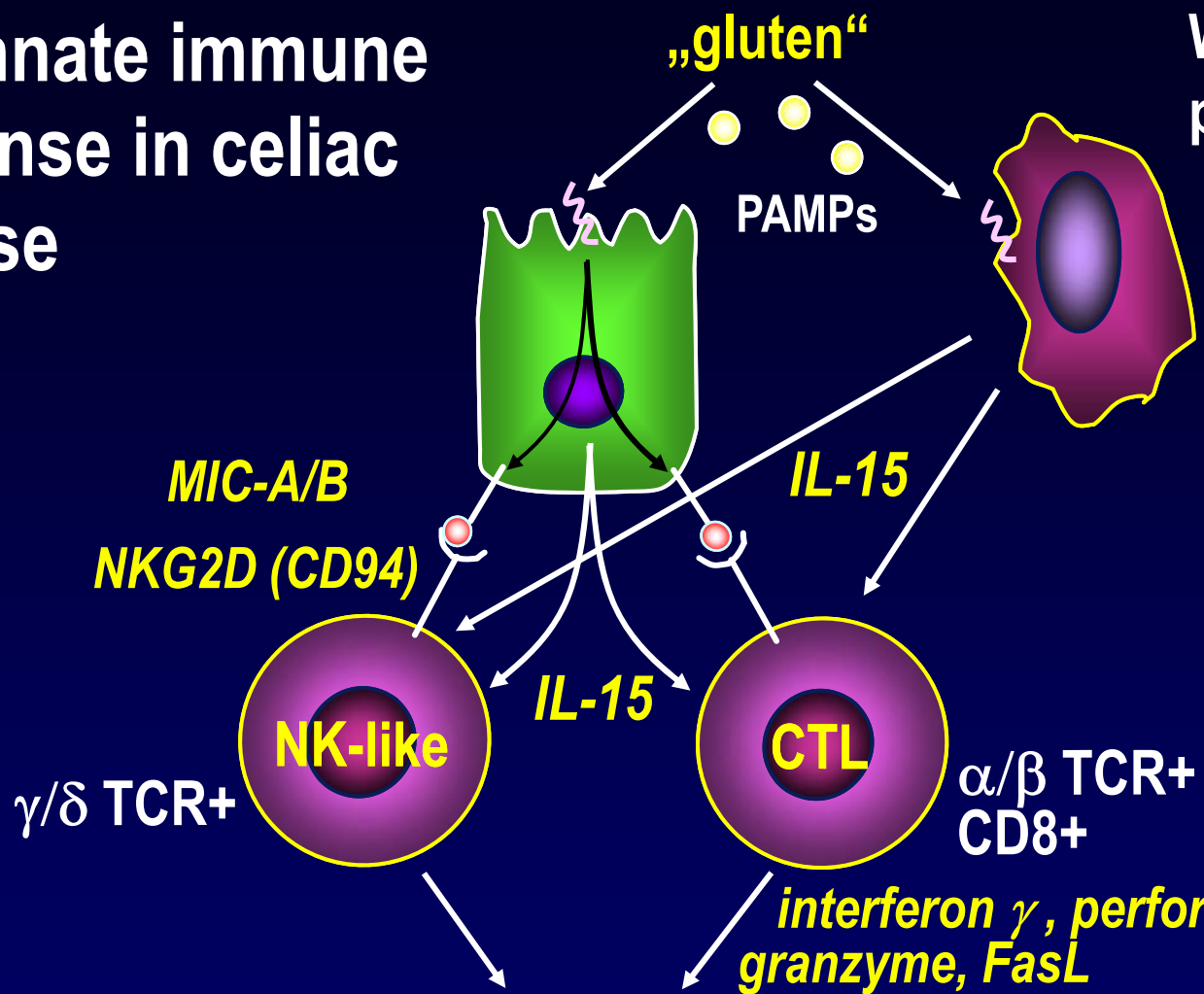
Role of the Innate Immune System in celiac disease – prior work

- Stimulation of biopsies from CD patients with PT gliadin or α2 gliadin p31-43 enhances IL-15 positive cells in the lamina propria *(Maiuri et al, Lancet 2003)*
- p31-43 induces MICA on intestinal epithelial cells via IL-15, serving as target for cytotoxic IELs *(Hue et al, Immunity 2004)*
- PT gliadin and different gliadin peptides induce activation & maturation of monocytes, macrophages & DCs *(Tuckova et al, J Leuk Biol 2002; Palova-Jelinkova et al, FEBS Lett 2004, J Immunol 2005; Nikulina et al, J Immunol 2004; Cinova et al, J Clin Immunol 2007; Rakhimova et al, J Clin Immunol 2008)*
- Gliadin enhances intestinal permeability and DC activation via MyD88 (CXCR3 on intestinal epithelial cells as gliadin receptor) *(Thomas et al, J Immunol, 2006; Lammert et al, Gastroenterology 2008)*

?

1. LPS contamination not strictly ruled out
2. No reproducible identification of a certain (set of) gliadin peptide(s)
3. No plausible receptor identified

The innate immune response in celiac disease



→ potentiation of the adaptive immune response to gluten

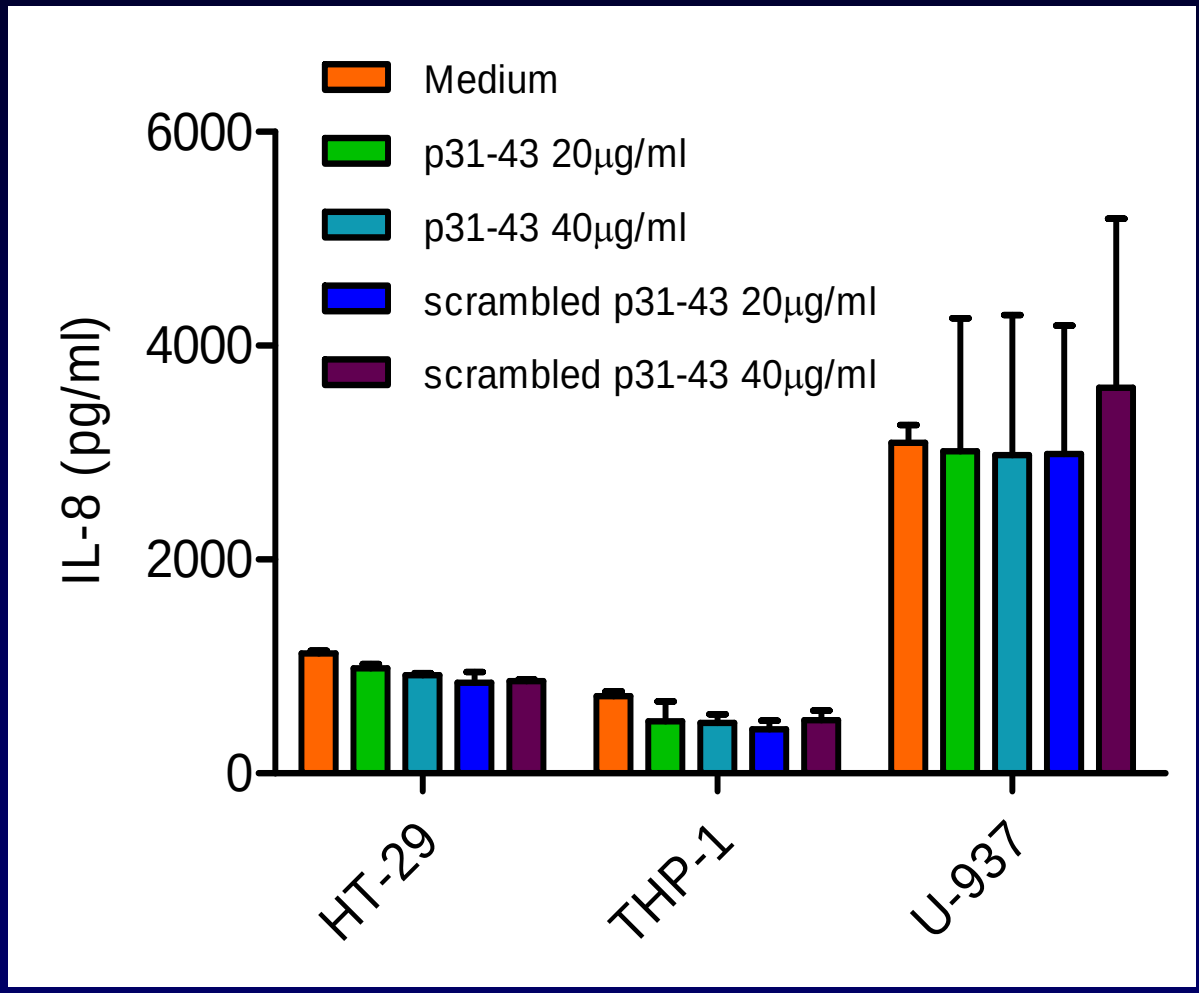
What about professional APC ?

Is it really gluten ?

IL-15
central growth factor for intraepithelial NK cells and CTL

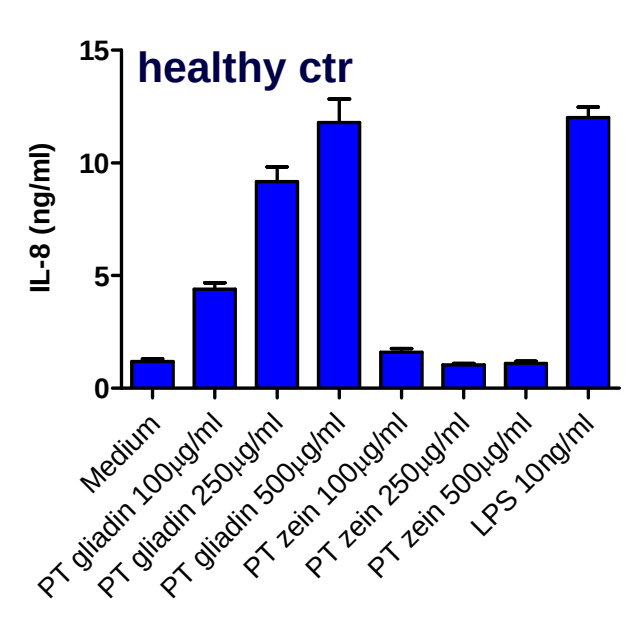
Jabri B et al, Gastroenterology 2000
Maiuri L et al, Lancet 2003
Hue S et al, Immunity 2004
Meresse B et al, Immunity 2004
Rakhimova M et al, J Clin Immunol 2008

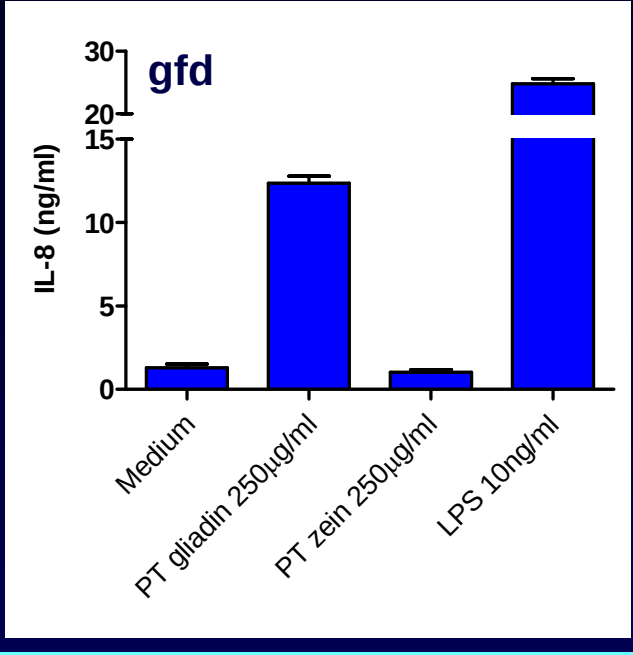
p31-43 and PT gliadin do not stimulate intestinal epithelial cells, monocytes, macrophages or DCs

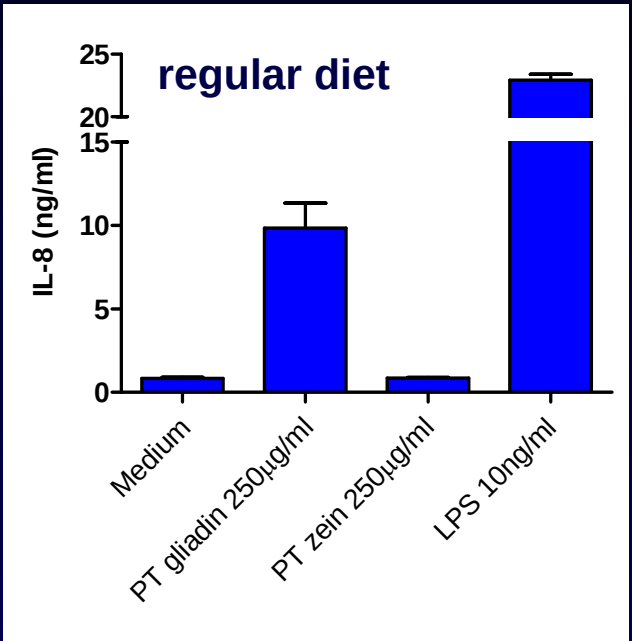


Junker et al, J Exp Med 2012

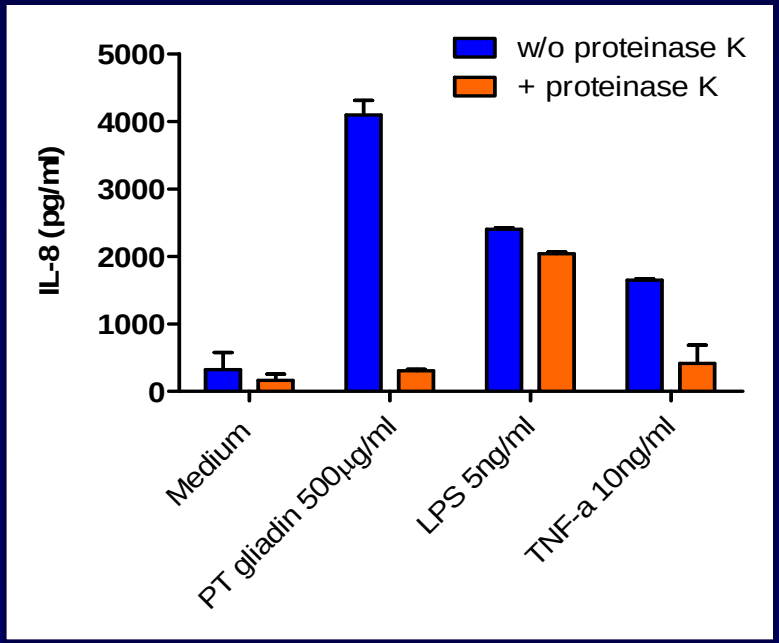
PT gliadin stimulates monocyte derived DCs from controls and celiac patients





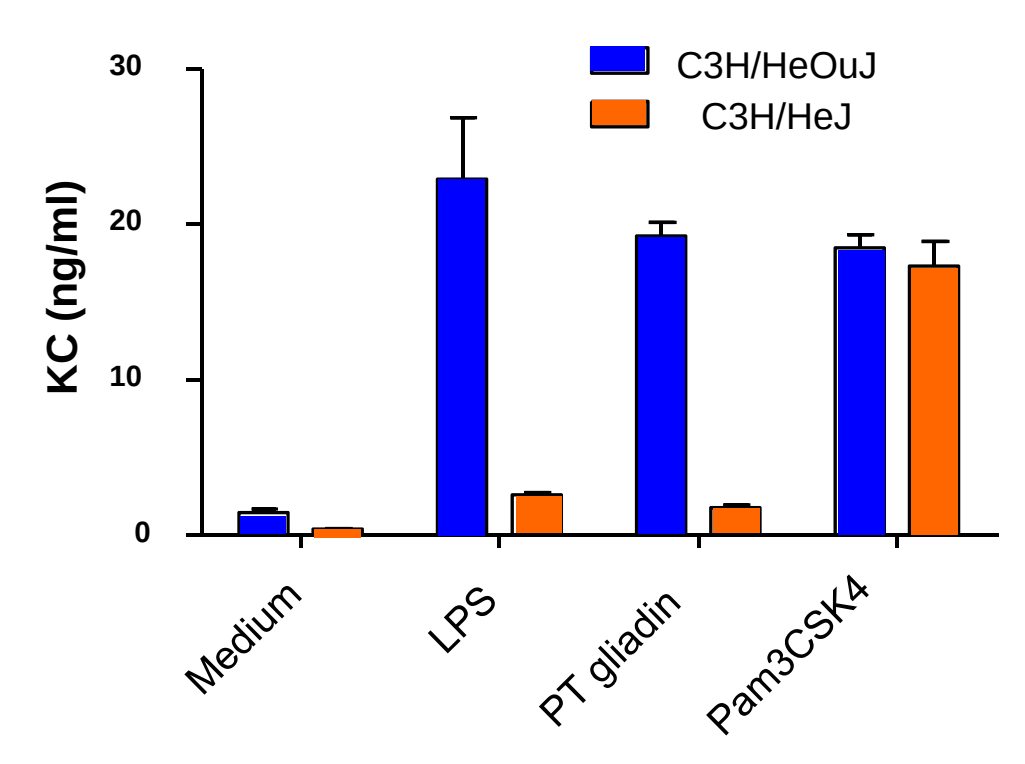


LPS is not a contaminant



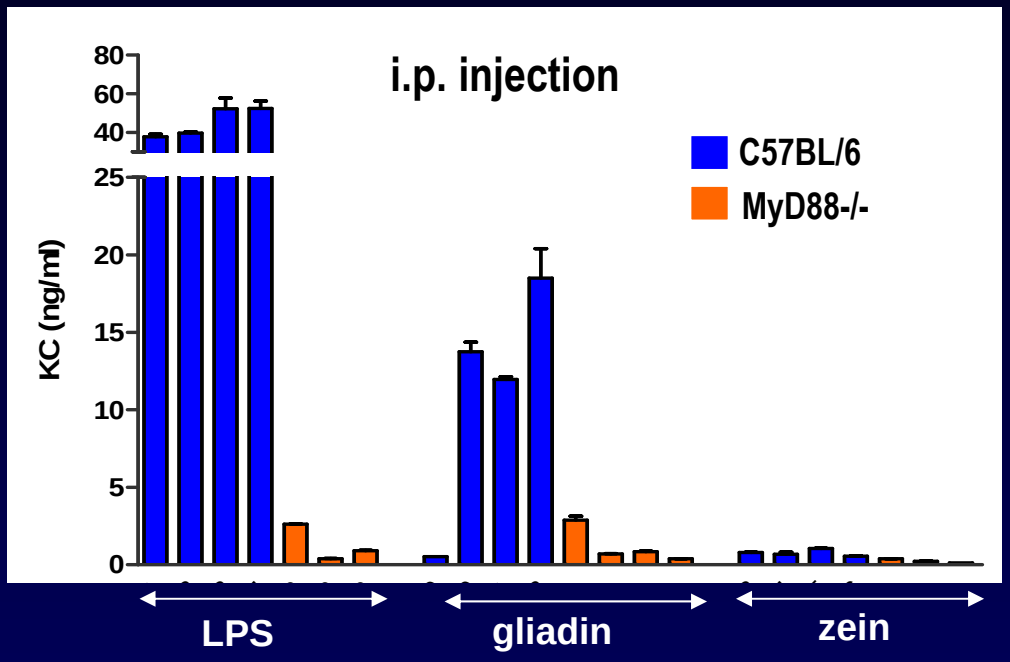
Junker et al, J Exp Med 2012

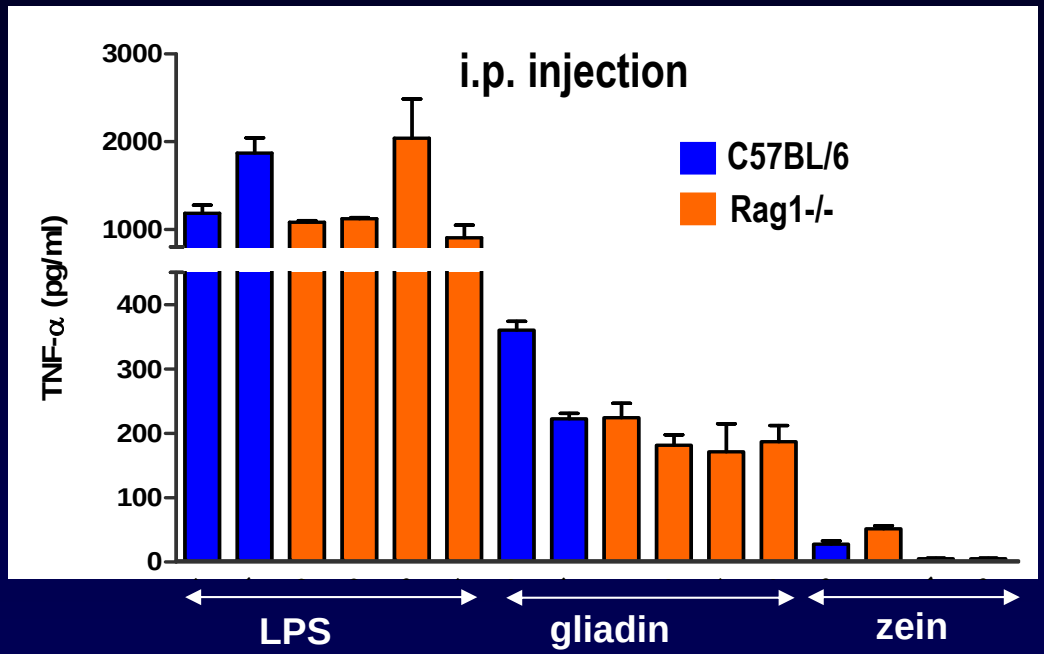
PT gliadin-induces innate immune responses via TLR4 in vitro and in vivo

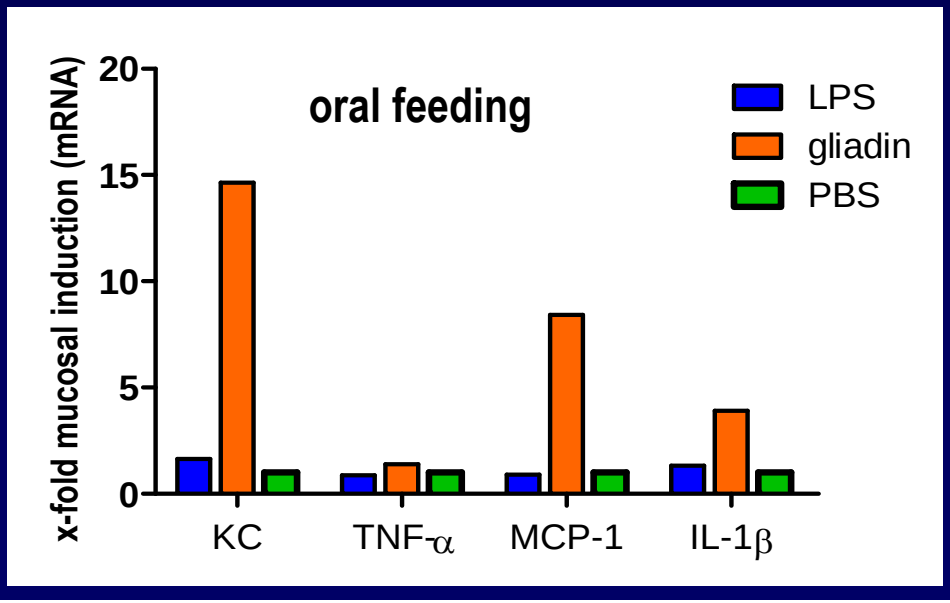


C3H/HeJ mice: TLR4 deficient due to a spontaneous point mutation

Gliadin mediated innate immune responses *in vivo*



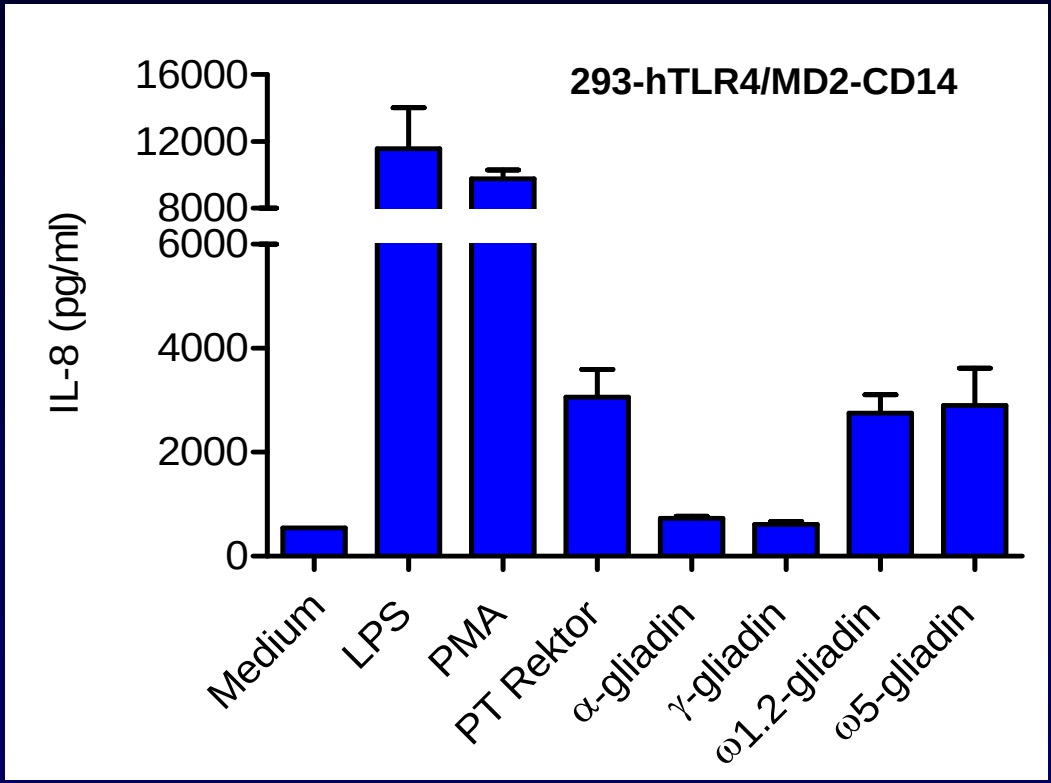


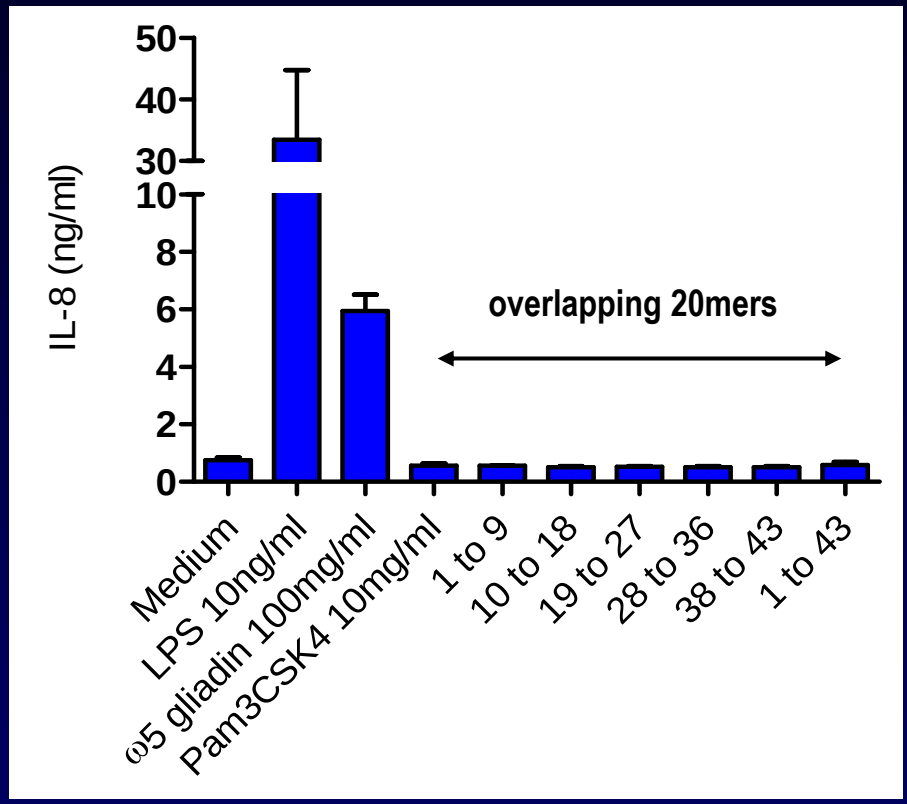


Oral LPS is inactivated by stomach acid and intestinal alkaline phosphatase, while the activity in gliadin is not

Junker et al, J Exp Med 2012

The activity is contained in the ω-gliadin fraction





Comparison of the gliadin fractions by SDS-PAGE showed a minor component of 15kDa associated only with ω-gliadins

There is a little hidden bird on this foto – find it !

Wheat amylase-trypsin Inhibitors (ATIs) trigger intestinal innate immunity in macrophages and dendritic cells via TLR4

Junker Y et al, J Exp Med 2012

```
CM3    MACKSSCSLLLLAAVLLSVLAAASASGSCVPGVAFRTNLLPHCRDYVLQQTCGTFTPGSK 60
0.19   ----------------------SGPWMCYPGQAFQVPALPACRPLLRLQCNGSQVPEAV 37
                              *..  * ** **:.  ** **  :  *  *: .* :

CM3    LPEWMTSASIYSPGKPYLAKLYCCQELAEISQQCRCEALRYFIALPVPSQPVDPRSGNVG 120
0.19   LRD-------------------CCQQLAHISEWCRCGALYSMLDSMYKEHGAQE-----G 73
       * :                  ***:**.**: *** **  ::     .: .:       *

CM3    ESGLIDLPGCPREMQWDFVRLLVAPGQCNLATIHN---VRYCPAVEQPLWI 168
0.19   QAGTGAFPRCRREVVKLTAASITAVCRLPIVVDASGDGAYVCKDVAAYPDA 124
       ::*   :* * **:    .  :.*  :  :..  .   .  *  *
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Oda Y et al, Biochemistry 1997

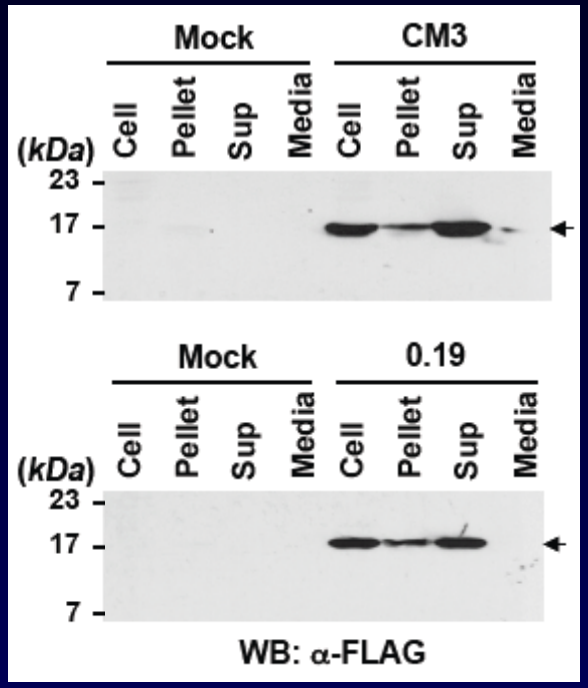
Characteristics und function of wheat ATIs

- Family of up to 11 similar, small and compact proteins
- 5(4) intramolecular SS-bonds, resistant to intestinal degradation
- Pest control (inhibition of parasite enzymes)
- Known major allergens of baker‘s asthma
- Content paralles that of gluten – association with omega-gliadins

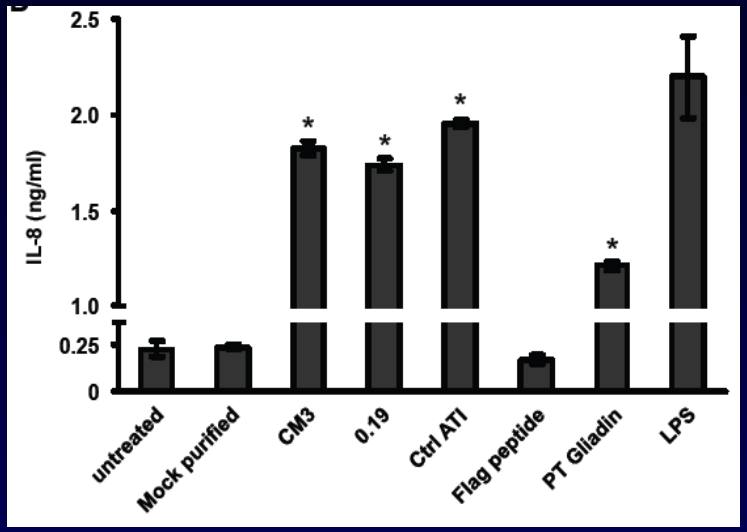
Tatham & Shewry, Clin Exp Allergy 2008

Zevallos VF et al, DDW 2012, #1309

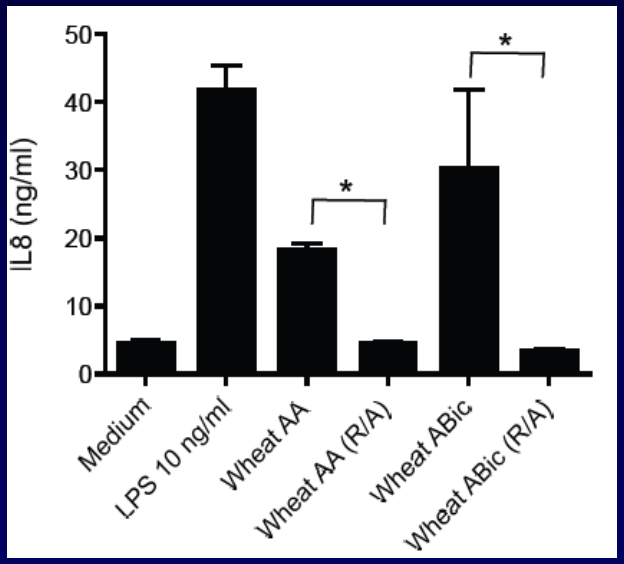
Activity of 2 major wheat ATIs expressed in eukaryotic cells



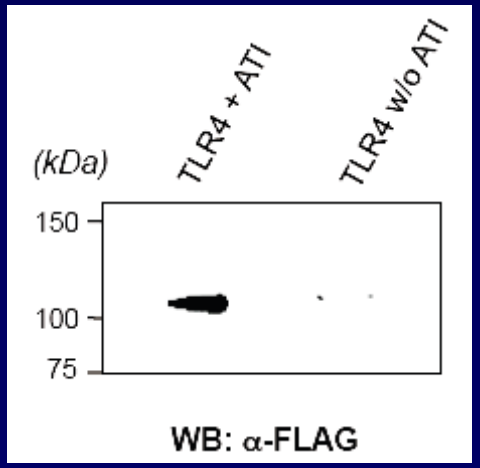
Activation of monocytes-macrophages



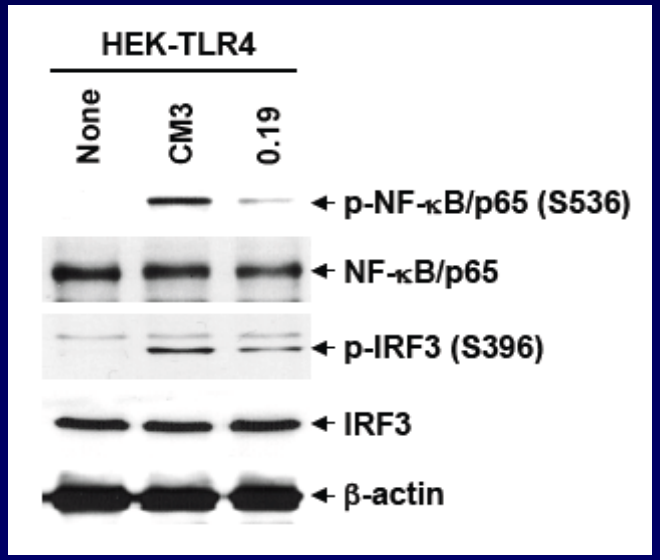
Inactivation by S-S reduction



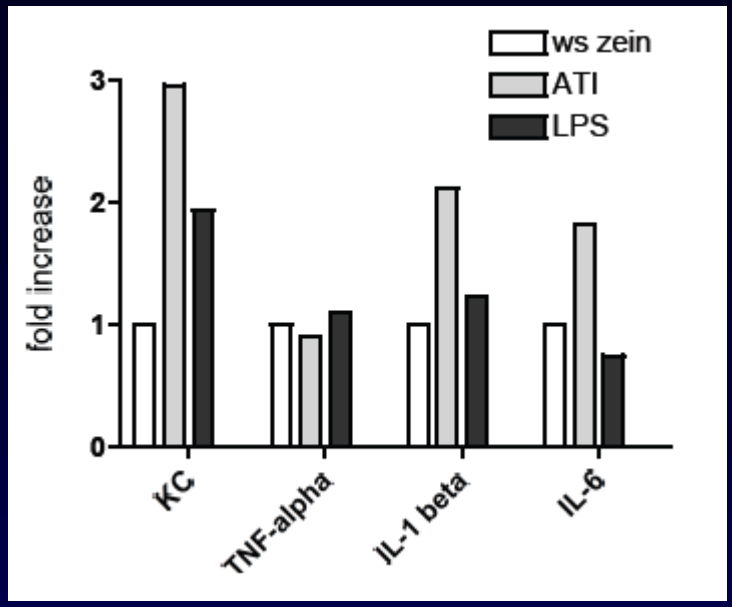
Physical interaction with TLR4



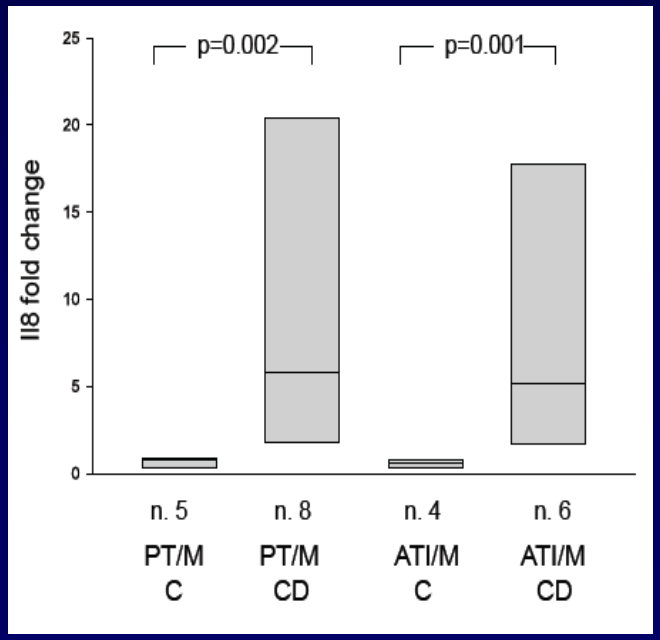
Activation of both TLR4 pathways

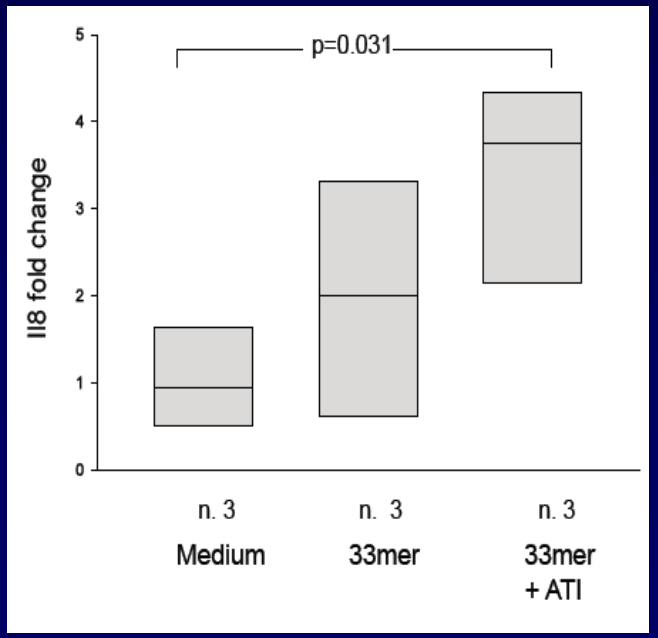


Oral feeding of ATI (50μg/mouse) causes low level intestinal inflammation



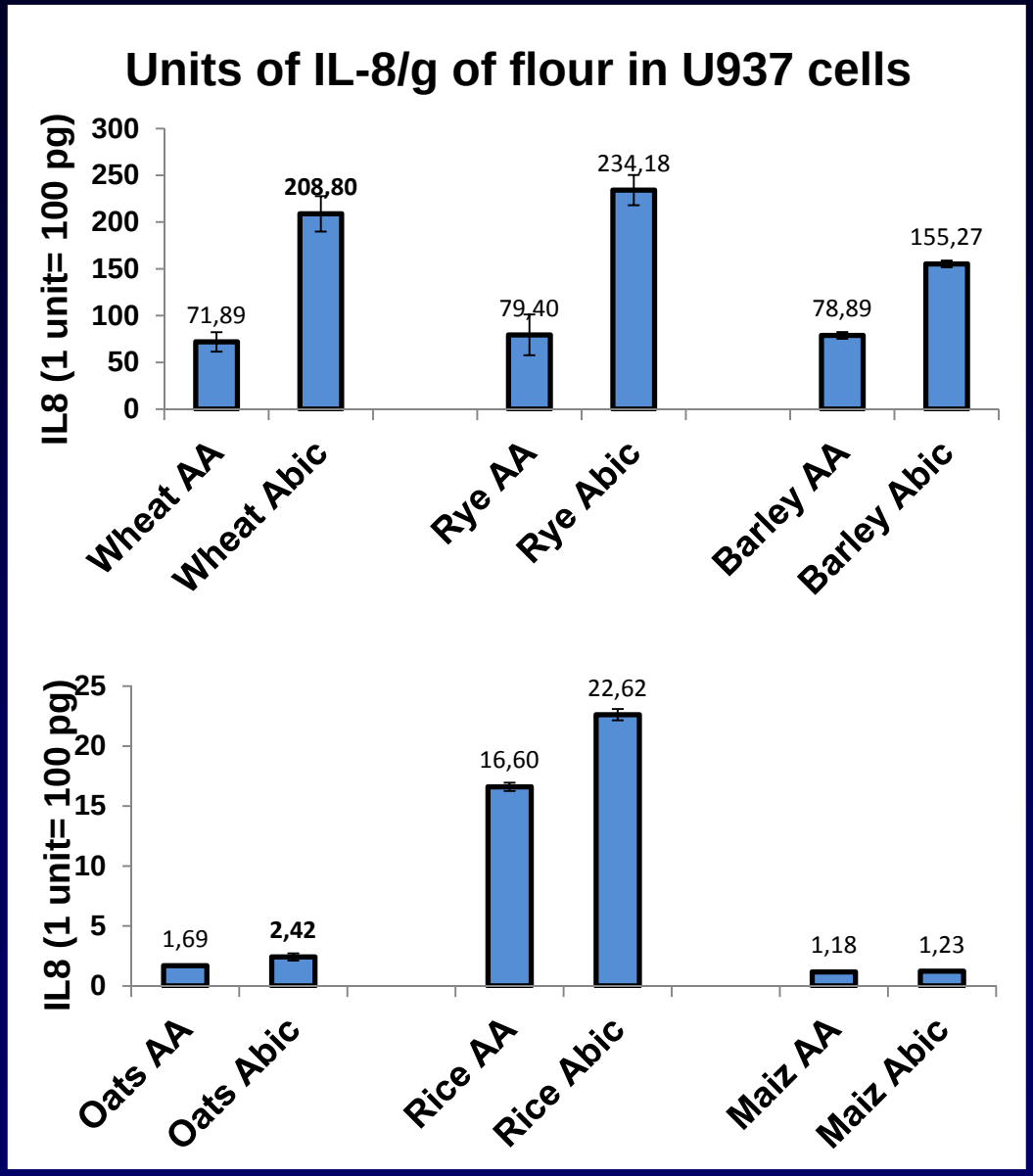
ATI promotes adaptive immunity in human CD biopsies





Junker et al, J Exp Med 2012

Classification of plants according to their relative potency to induce innate immunity



High: gluten containing

Wheat	*Triticum* aestivum
Barley	*Hordeum vulgare* L.
Rye	*Secale cereale*

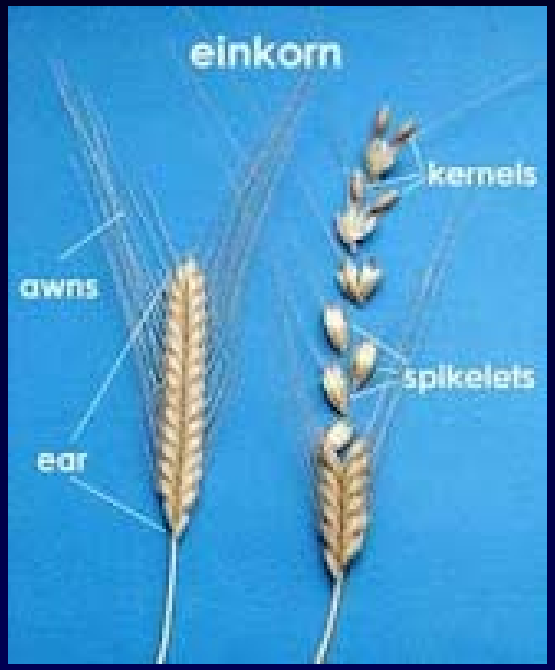
Medium: gluten-free (gluten-poor)

Soya	*Glycine Max*
Quinoa	*Chenopodium* quinoa
Buckwheat	*Fagopyrum esculentum*
Peas	*Pisum sativum*
Early Crops	*Einkorn<Emmer<Spelt*

Low: gluten free

Rice	*Oryza sativa*
Millet	*Panicum miliaceum*
Oats	*Avena sativa*
Maize	*Zea mays* L.
Amaranth	*Amaranthus caudatus*

Zevallos VF et al, unpublished



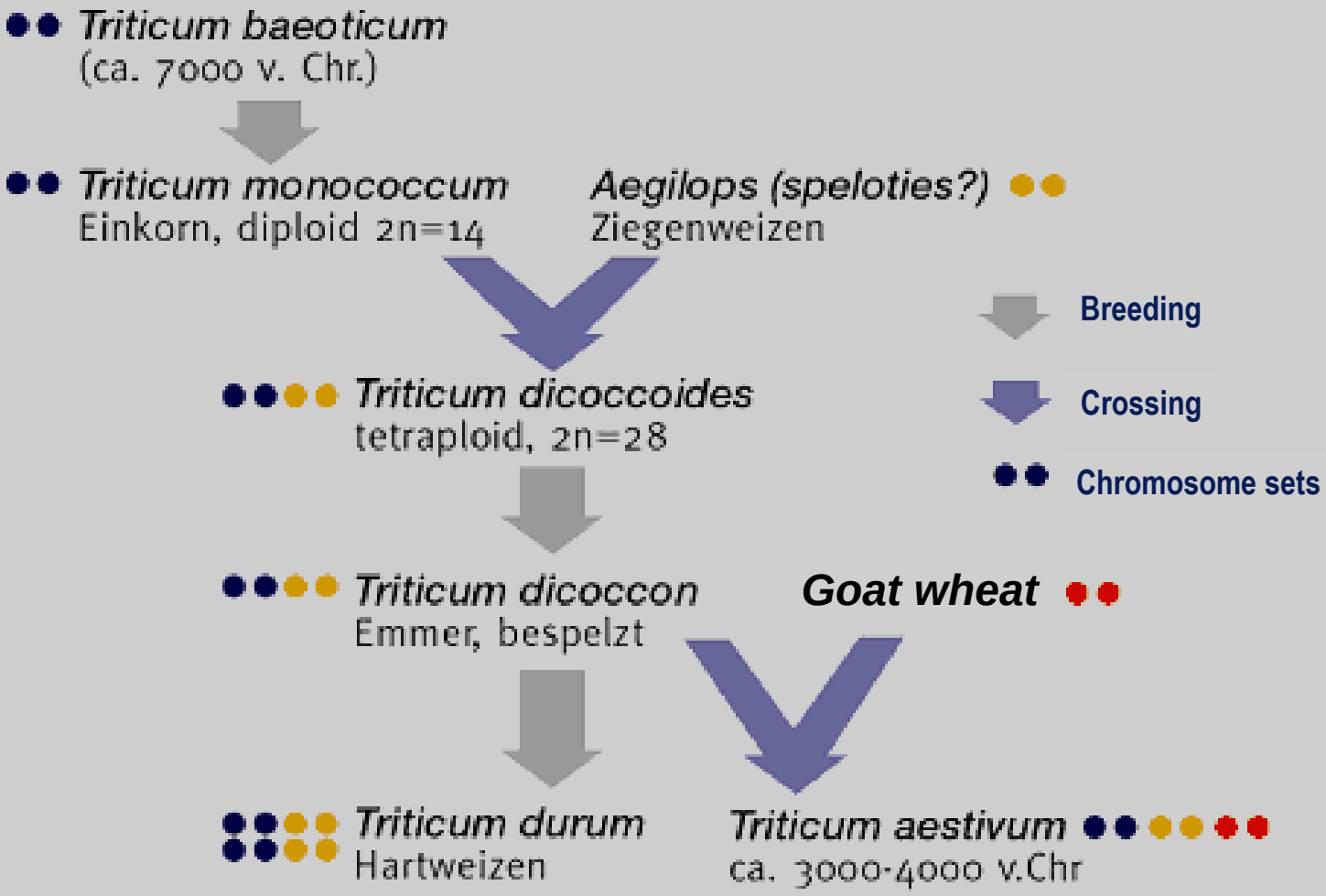
Einkorn

Emmer

Modern wheat

Spelt

Origin of Wheat



Increase of content of immunogenic gluten epitopes and ATIs with resistance breeding and higher ploidity

Breeder‘s dilemma

Breeding for high yield and high pest resistance

- **Reduction of nutritional value (essential amino acids, minerals, vitamins)**
- **Increase in pest resistance proteins/ molecules potentially harmful to man**

Morris CE and Sands DC, Nat Biotechnol 2006

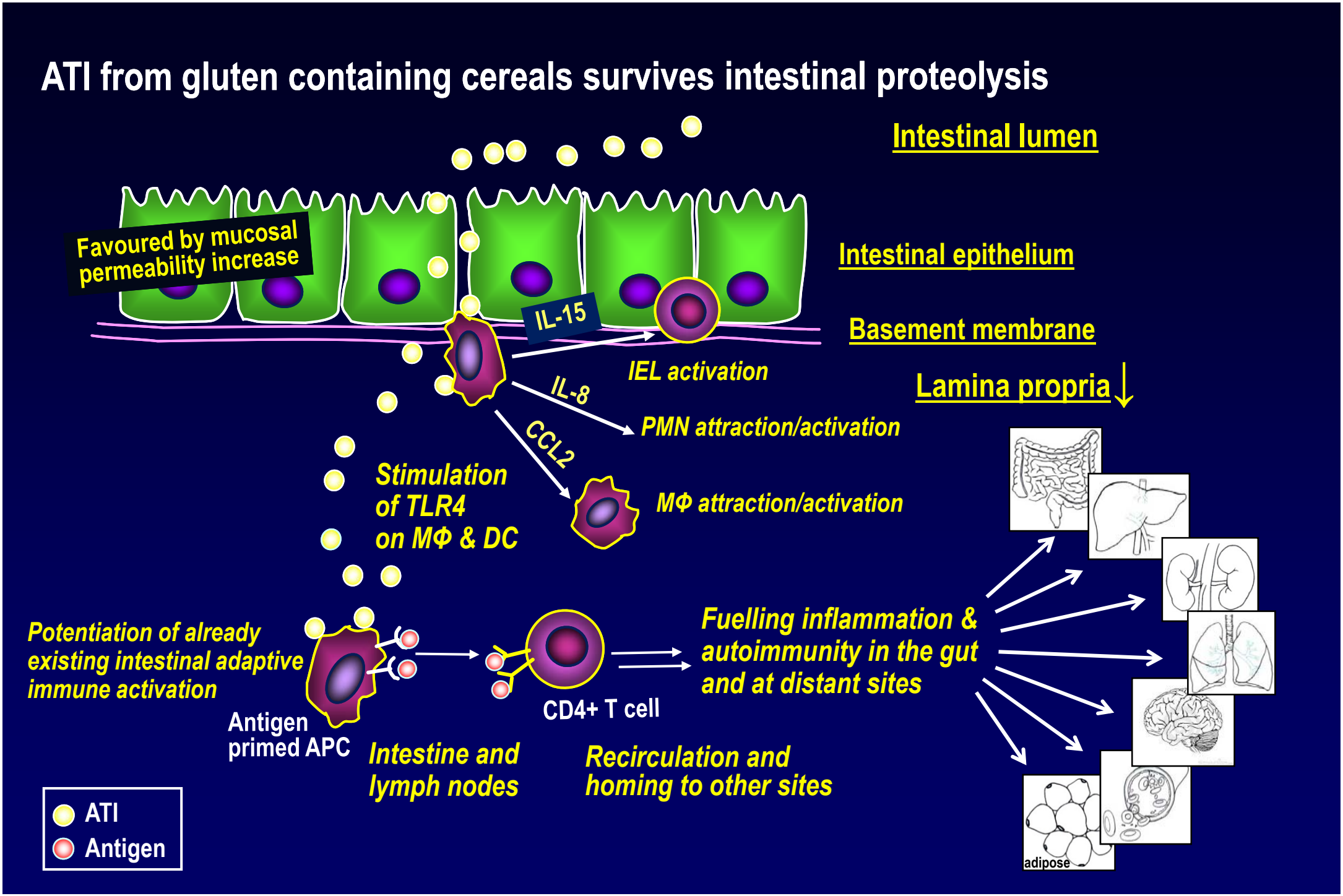
Non-celiac „gluten“ sensitivity

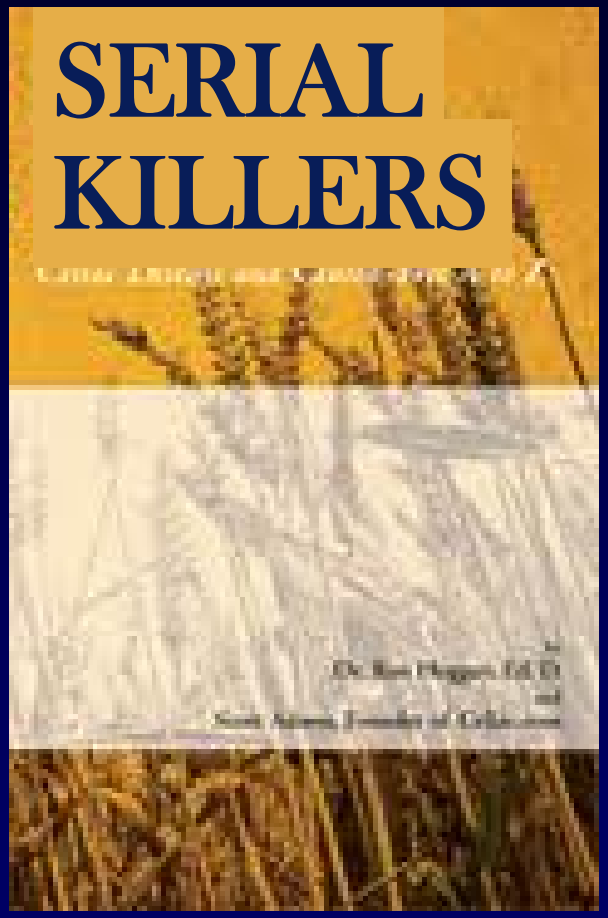
„Gluten sensitivity“ without overt small intestinal damage or auto-Abs

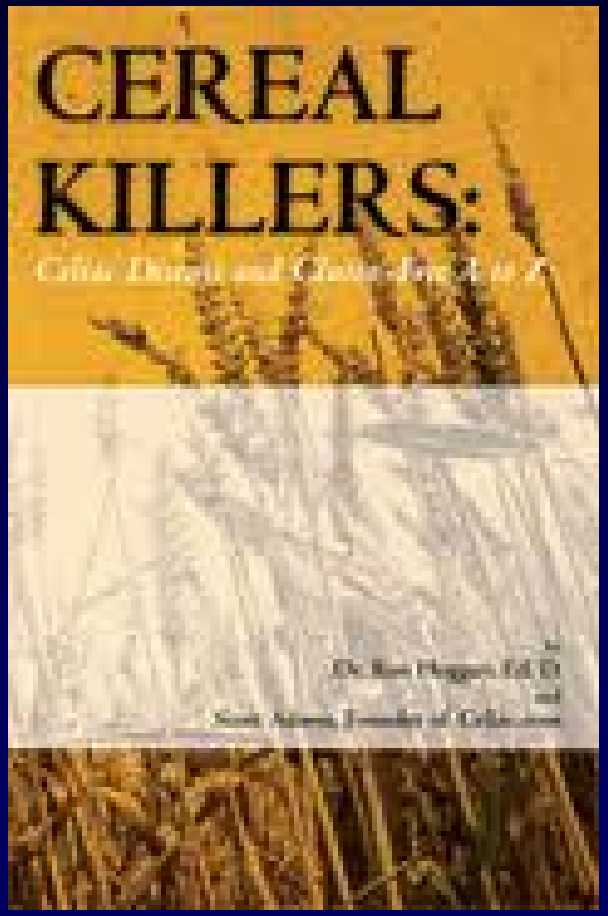
Estimated prevalence 3-10%
Role of ATIs ?

Studies in IBS, autoimmune-, metabolic, cardiovascular…… diseases are warranted

ATI from gluten containing cereals survives intestinal proteolysis




SERIAL
KILLERS


CEREAL
KILLERS:

Innate Immunity in Celiac Disease

- The mechanism by which certain gluten peptides (p31-43) may elicit innate mechanisms in biopsies ex vivo remains to be defined
- ATIs of gluten containing grains are strong inducers of innate immunity in DCs > macrophages > monocytes via TLR4
- Ingested ATIs induce low level intestinal inflammation in vivo
- ATI content of modern wheat has increased due to resistance breeding
- ATIs of gluten free foods have much less stimulatory activity
- Innate immunity to ATIs likely impacts other intestinal and non-intestinal inflammatory diseases

Research and Clinical Team

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