Istituto di Scienze dell'Alimentazione,
CNR Avellino

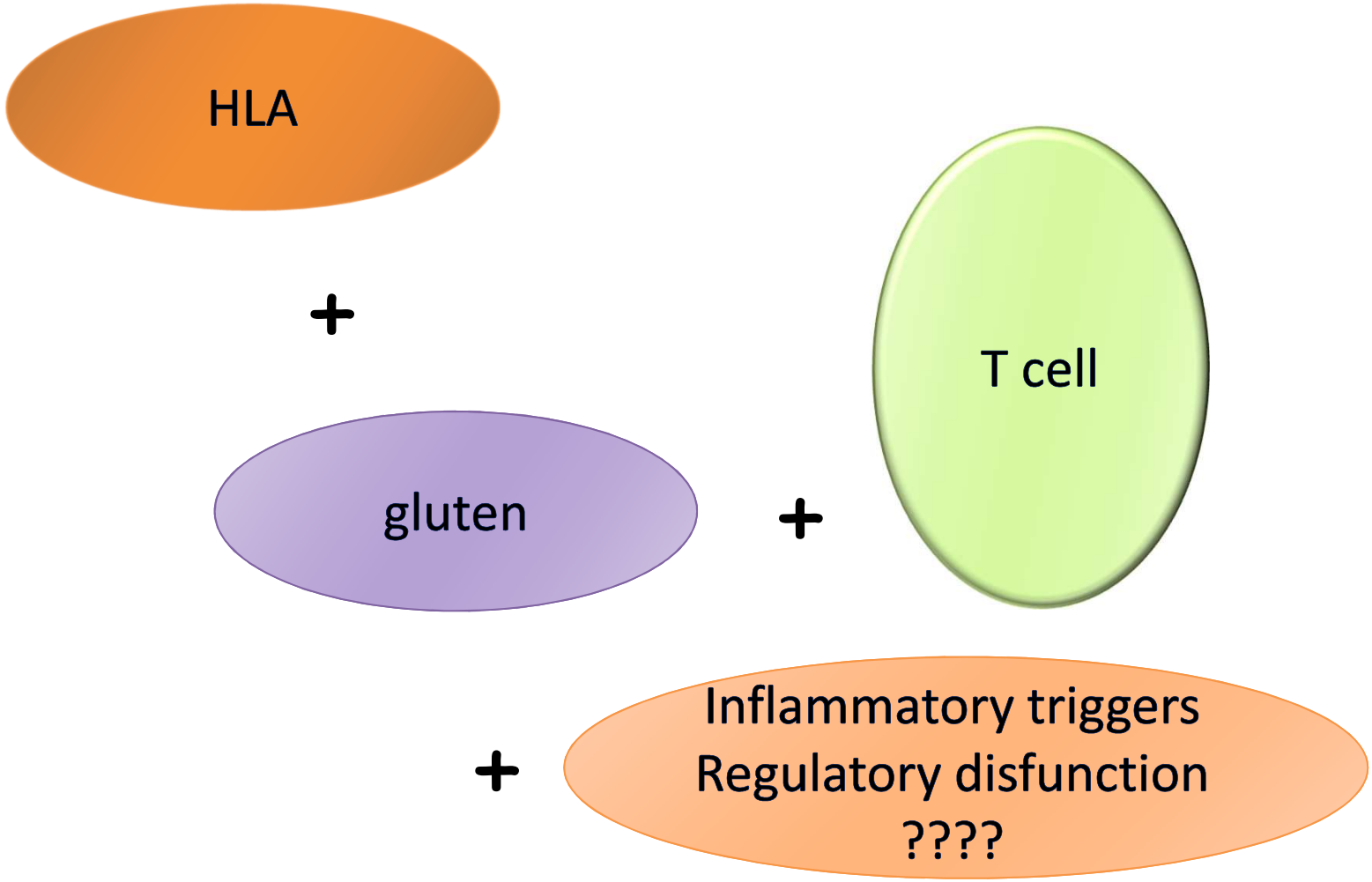
Prospettive di terapia

Carmen Gianfrani

Corso di Aggiornamento
La Celiachia nel terzo millennio: dalla diagnosi alla terapia
Caserta 5 Maggio 2012

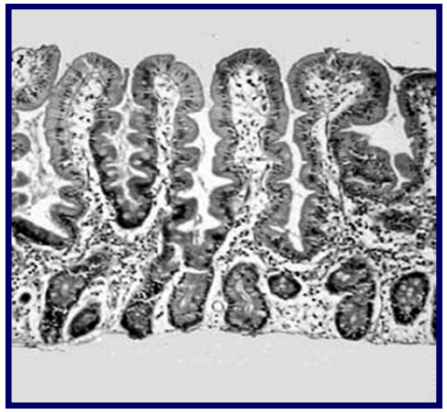
ELFID
European Laboratory for the investigation of Food-Induced Disease

Both genetic and environmental factors are involved in CD



Role of HLA CLASS II in CD

95% of Celiacs are DQ2 and/or DQ8



Normal jejunal mucosa

gluten

\+

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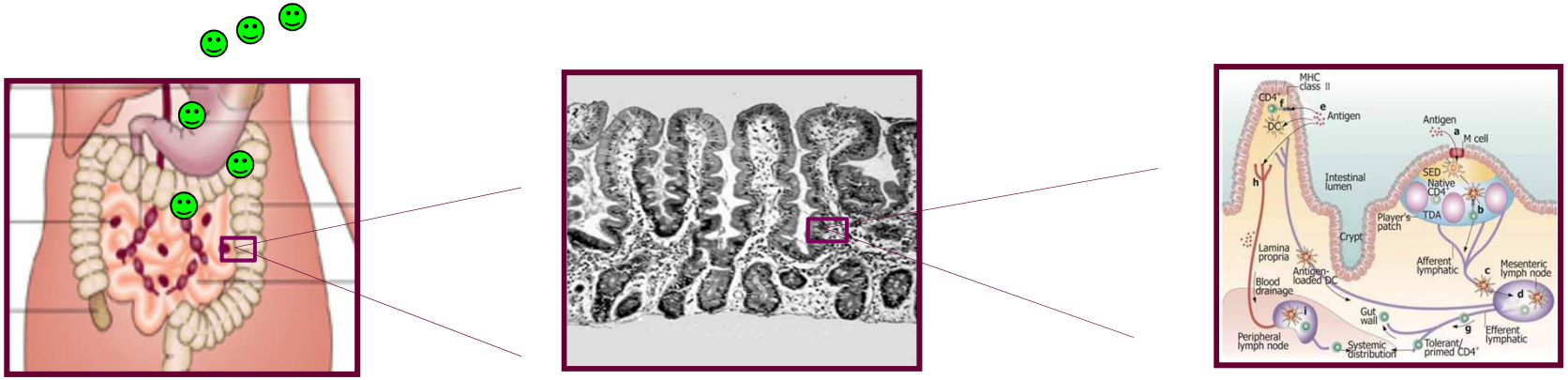
Atrophic mucosa

95% of DQ2/DQ8 positive individuals are NOT Celiacs

Oral Tolerance

In healthy jejunal mucosa food antigens and commensal flora are beneficial and tolerated

- food antigens
- commensal bacteria



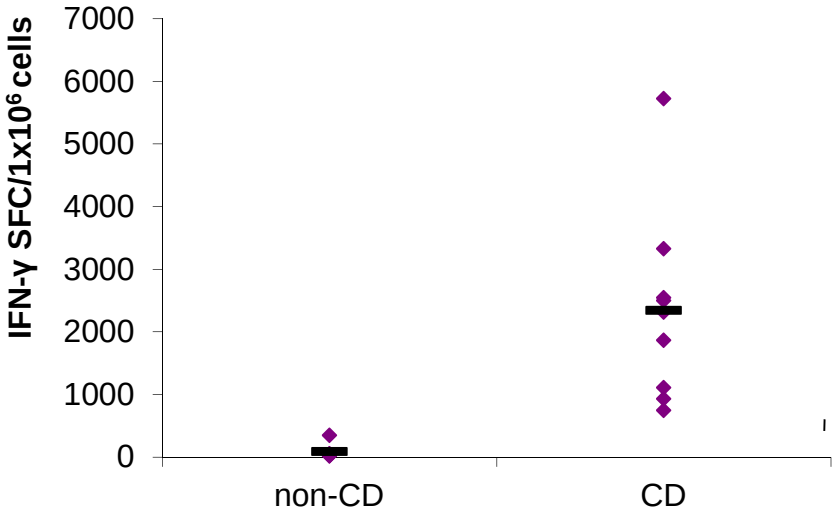
Mowat, Nat Rev Immunol 2003

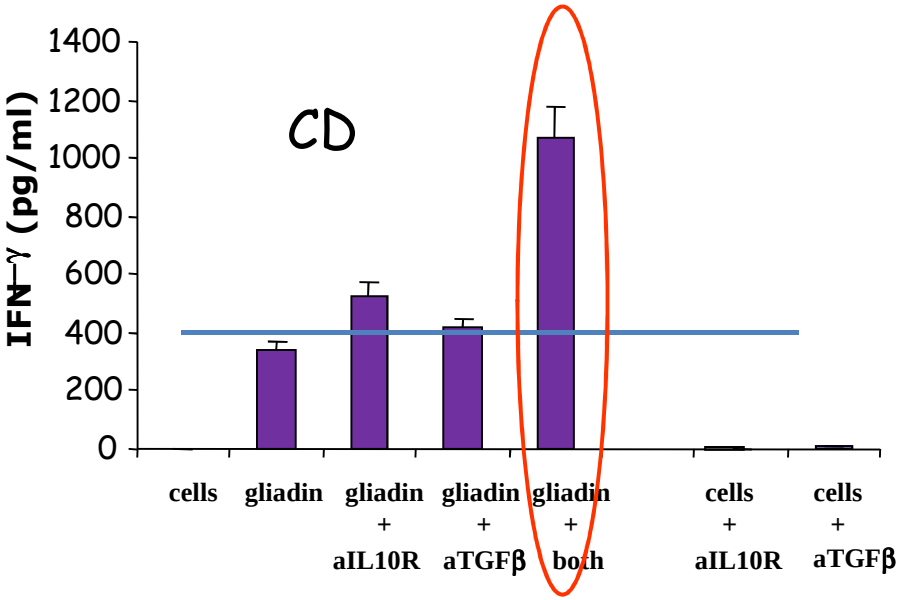
Oral Tolerance is a tightly controlled immune phenomenon.
The intestinal immune system has to discriminate between **harmful** and **beneficial** luminal antigens

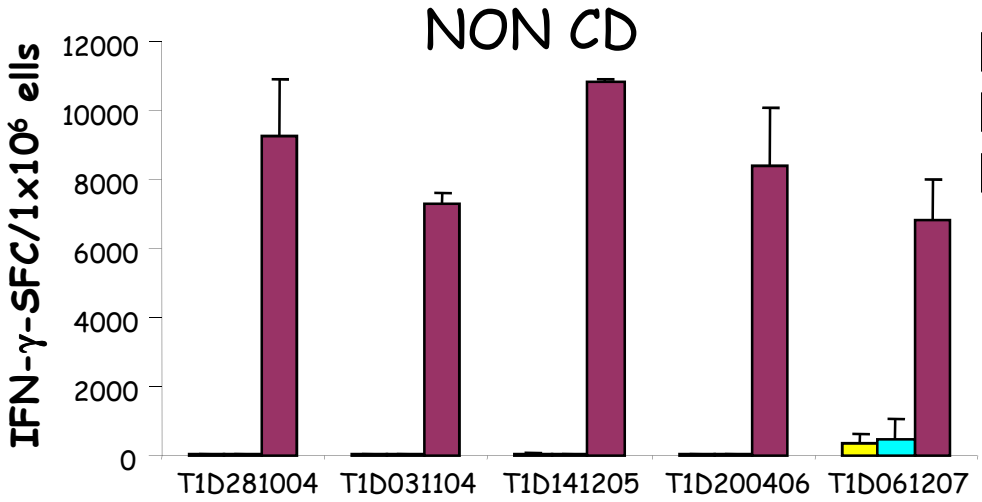
Key role of Regulatory T cells and cytokines IL10 and TGFβ

Weiner Immunol Rev. 2011
Powrie, Immunol Rev. 2006

Inflammatory response to gluten occurs only in CD and is «regulated» by IL10 and TGF-β







gliadin

gliadin + α-IL-10R and α-TGFβ

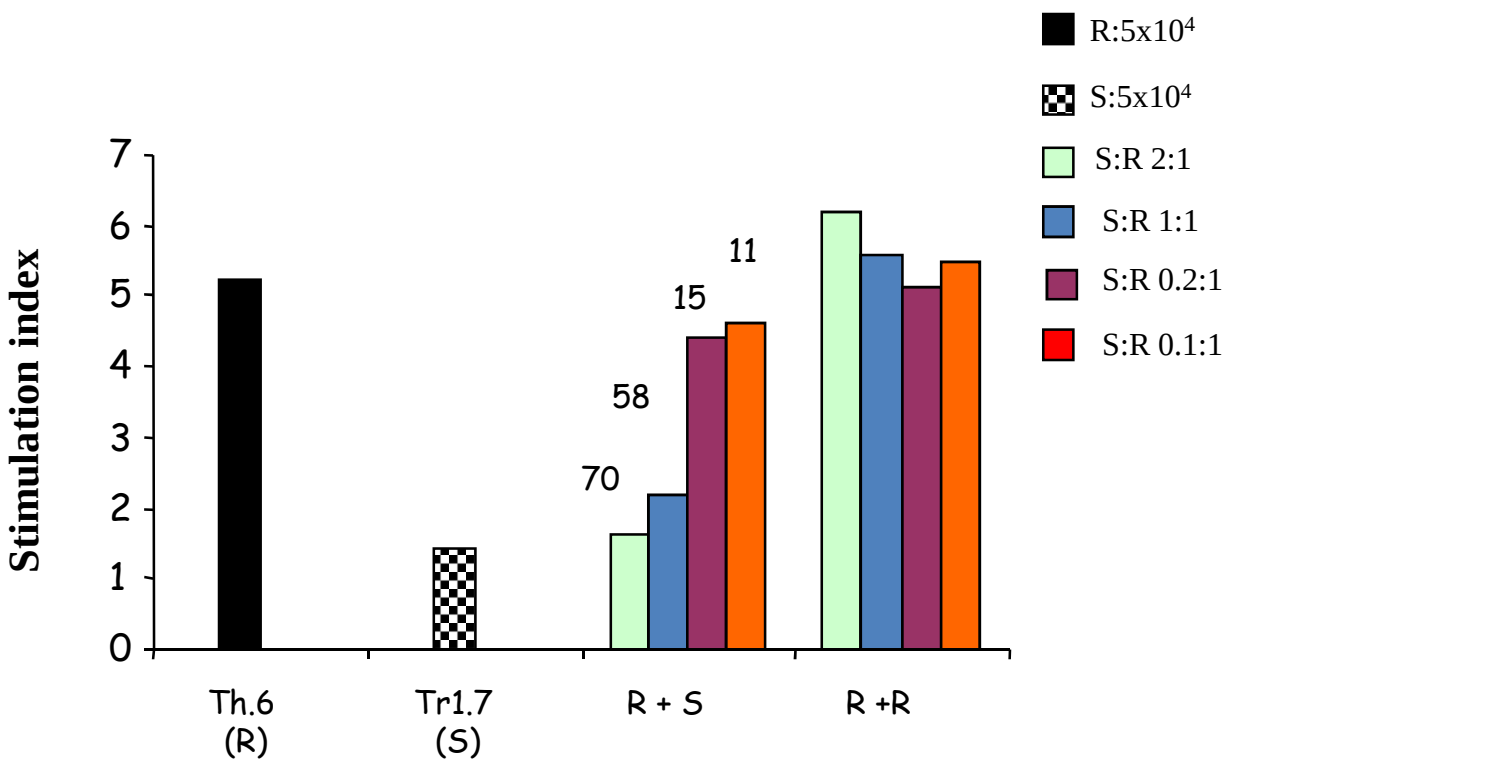
PHA

Gianfrani C. et al J Immunol 2006
Gianfrani C unpublished

Gliadin-Specific Type 1 Regulatory T Cells from the Intestinal Mucosa of Treated Celiac Patients Inhibit Pathogenic T Cells

Carmen Gianfrani,[1]* Megan K. Levings,[2†‡] Claudia Sartirana,[†] Giuseppe Mazzarella,* Gianvincenzo Barba,* Delia Zanzi,[‡] Alessandra Camarca,*[‡] Gaetano Iaquinto,[§] Nicola Giardullo,[§] Salvatore Auricchio,[‡] Riccardo Troncone,*[‡] and Maria-Grazia Roncarolo[†¶]

The Journal of Immunology, 2006, 177: 4178–4186.



Standpoint:

activation of both pro-inflammatory and regulatory pathways in CD mucosa

gluten

Pro-inflammatory Immune Response

✓Proliferation/cytotoxicity

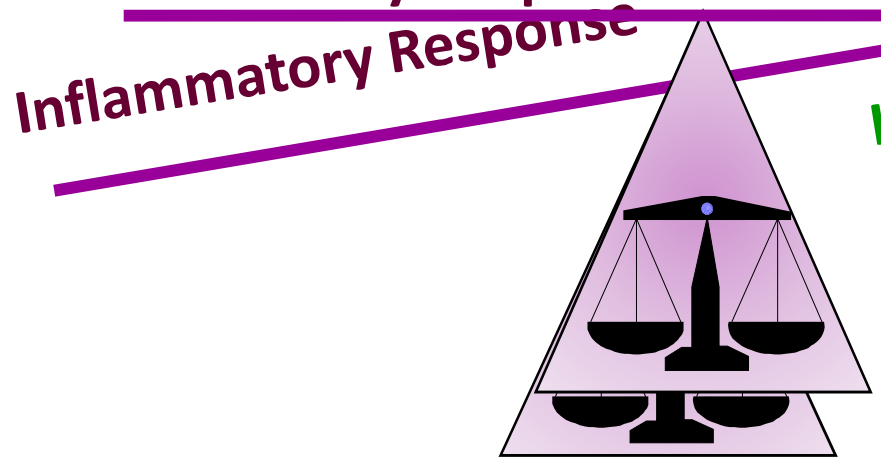
✓IFNγ, TNFα, IL-21

gluten

Regulatory Immune response

✓IL10+TGFβ+ Tr1

✓CD4+CD25+Foxp3+



Treg-based therapy

Is Treg-based therapy suitable in celiac disease?

Possible strategies:

i. In vitro induction/expansion of gut homing Tr1 and delivery into patients.

ii. Orally administration of genetically modified bacteria (IL-10/gliadin secreting lactococcus lactis), to *in vivo* induce or expand Tr1 cells

Proof of concept status

ImmusanT
DOMINANCE IN IMMUNOTHERAPY

Vaccination for Celiac Disease: utopia or concrete hope for Celiac Disease recovery

Courtesy of Bob Anderson

ImmusanT, Inc • One Broadway, 14th Floor • Cambridge, MA 02142

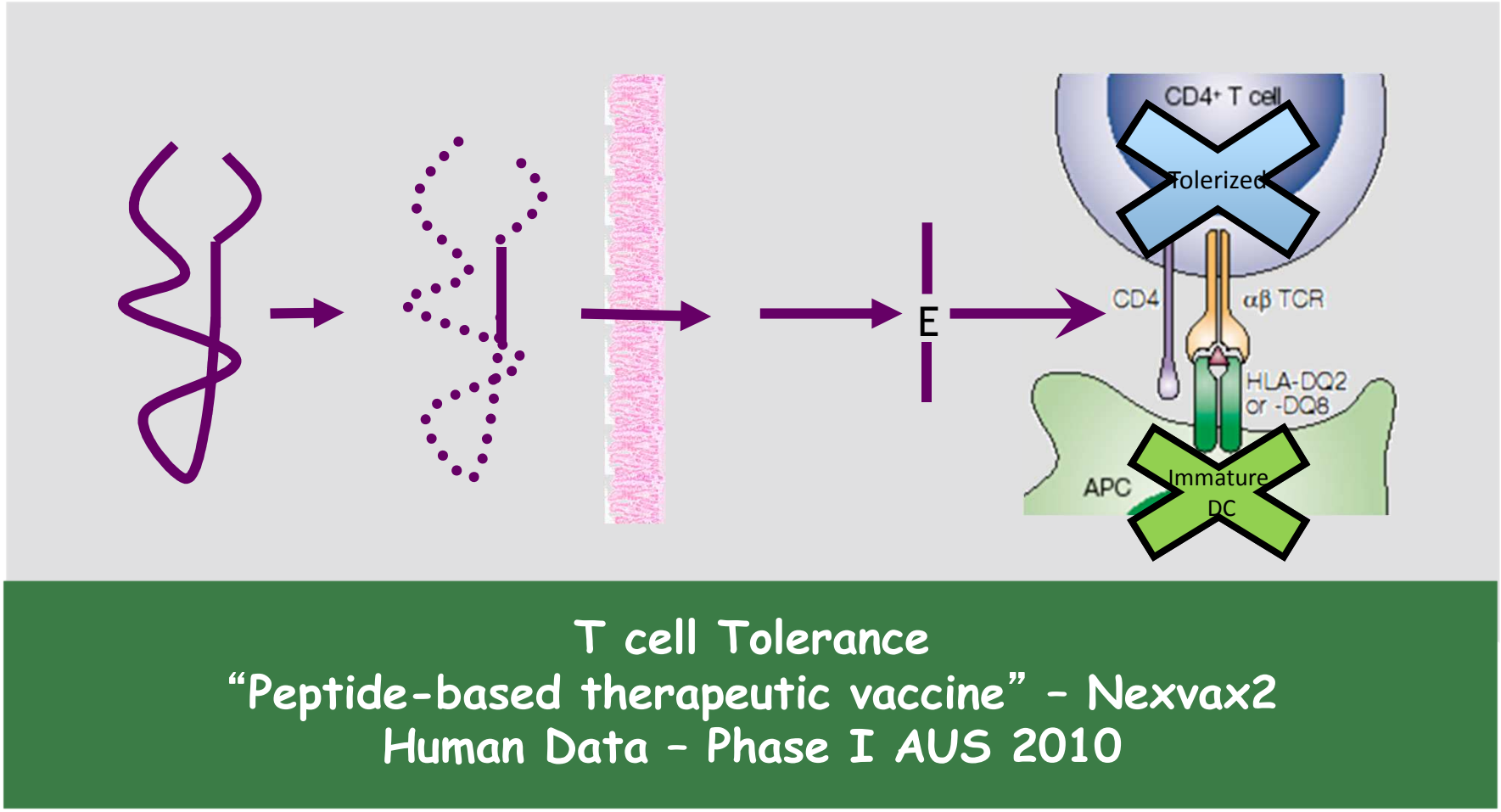
Key Points

- Objective: Treatment of celiac disease without gluten free diet
- Design and development of a tolerizing peptide immunotherapy
- Composition: Validity of peptide selection
- Proof of concept: Tolerizing T cells in transgenic mice
- Clinical trial – Phase 1

Courtesy of Bob Florence 2012

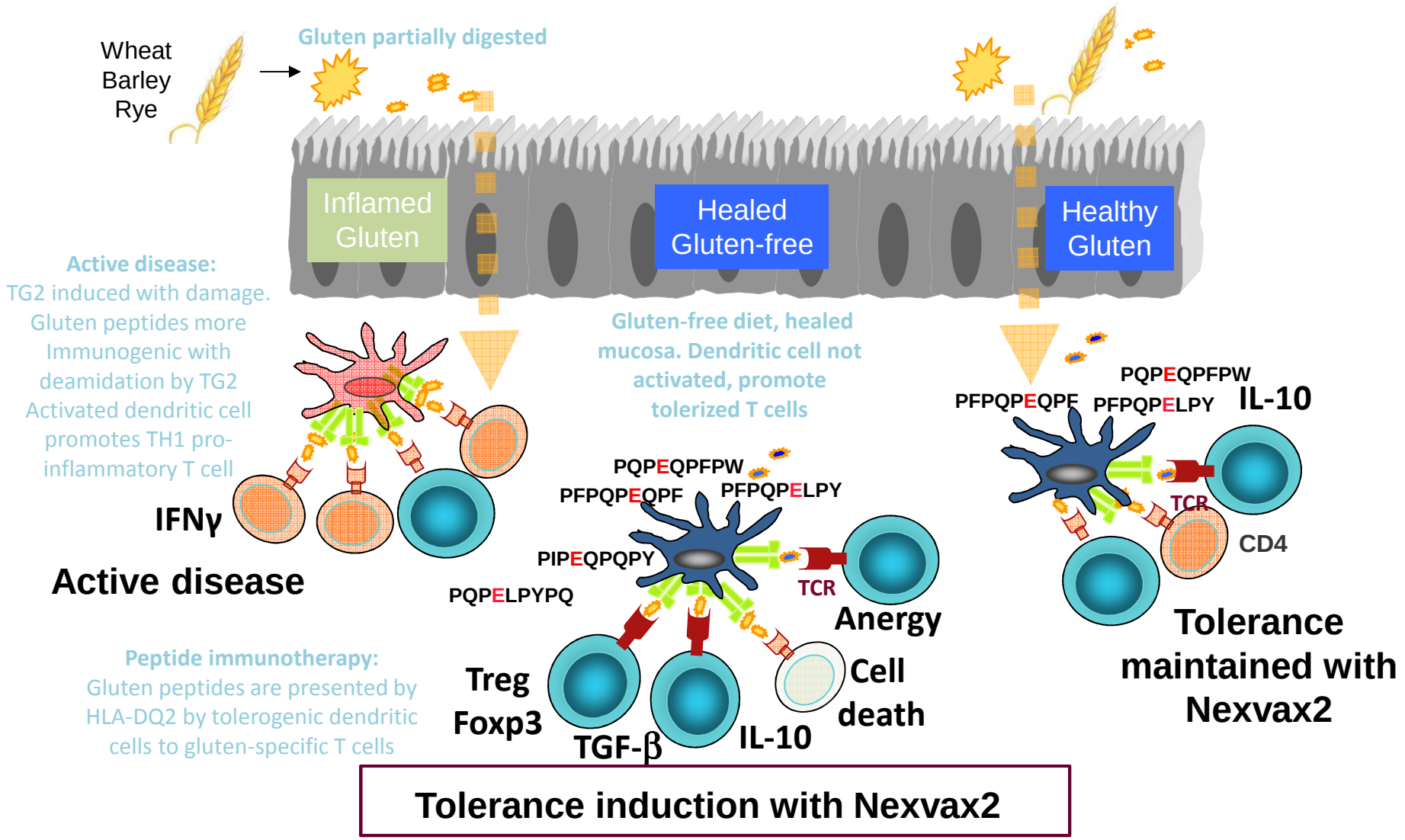
Peptide-based Immunotherapy for Celiac Disease:

Immuno-dominant peptides, T cells and tolerogenic dendritic cells



Courtesy of Bob Anderson

Use presentation of gluten peptides to delete gluten specific T cells or render them tolerogenic

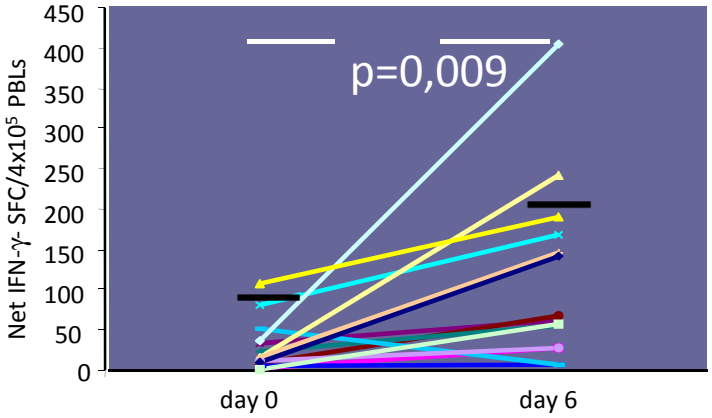


Courtesy of Bob Anderson

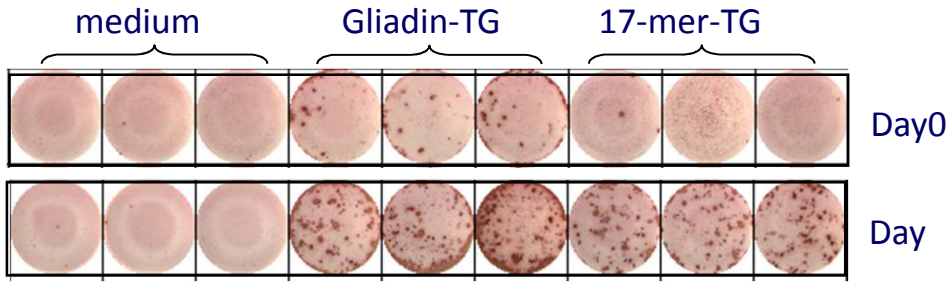
Strategies to identify «toxic» gluten peptides immunostimulatory for celiac patients to be included in a therapeutic vaccine

Gluten reactive T cells in the blood after a brief in vivo gluten challenge

- Patients on gluten-free diet consumed for 3 days 200gr of wheat bread
- Peripheral blood mononuclear cells are obtained at day0 and day6 after the commencing of gluten challenge
- IFN-γ ELISPOT after 36-40hr in vitro stimulation with deamidated gliadin or gliadin peptides



IFN-γ ELISPOT



Anderson et al. Nature Medicine 2000,
Camarca et al. Clin Exp Immunol 2012 in press

Immunodominant gluten peptides active after consuming wheat, barley and rye

RESEARCH ARTICLE

CELIAC DISEASE

Comprehensive, Quantitative Mapping of T Cell Epitopes in Gluten in Celiac Disease

Jason A. Tye-Din,[1,2,3]* Jessica A. Stewart,[1]* James A. Dromey,[1]* Tim Beissbarth,[1]*†
David A. van Heel,[4] Arthur Tatham,[5] Kate Henderson,[6] Stuart I. Mannering,[1]‡ Carmen Gianfrani,[7]
Derek P. Jewell,[8] Adrian V. S. Hill,[9] James McCluskey,[10] Jamie Rossjohn,[6] Robert P. Anderson[1,3]§

(Published 21 July 2010; Volume 2 Issue 41 41ra51)

www.ScienceTranslationalMedicine.org 21 July 2010 Vol 2 Issue 41 41ra51

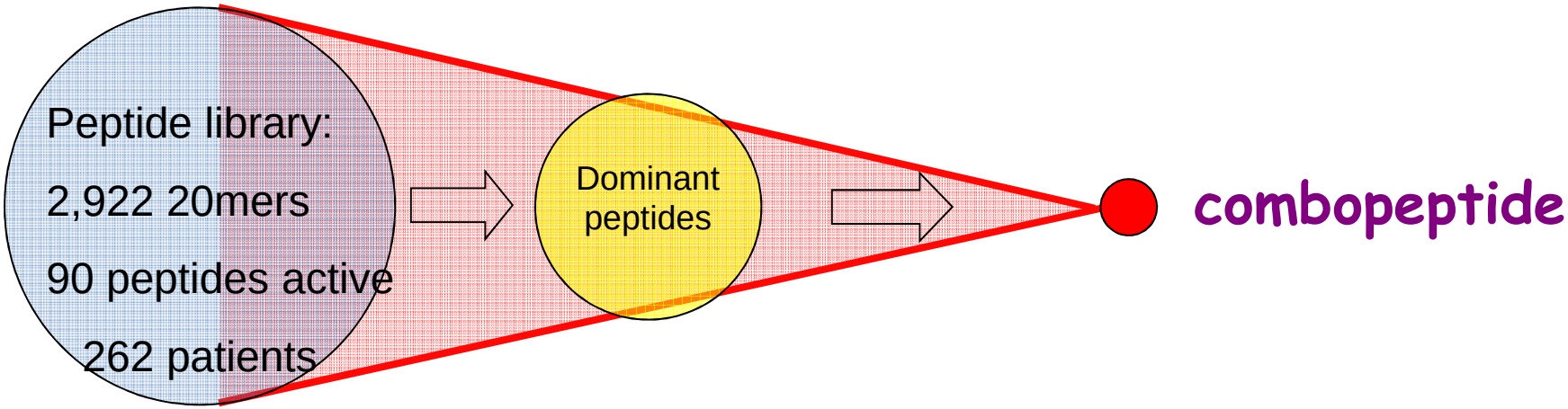
Dominant stimulatory gluten peptides are in wheat, barley and rye

	Wheat	Barley	Rye
α-gliadin PFPQPELPYPQ	✔		
ω-gliadin PFPQPEQPFPW	✓	✔	✔
B/C-Hordein PIPEQPQPY		✔	
ω-secalin PFPEQPEQI			✓

Tye-Din JA, Stewart JA, Dromey JA, et al. Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. *Sci Transl Med. 2010 21;2:41ra51.*

Courtesy of Bob Anderson

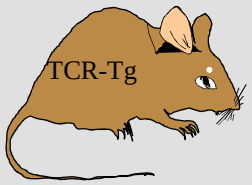
A large peptide library tested for toxicity in adult celiac patients



- DQ2-α-I/II
- DQ2-ω-I
- DQ2-Hor 1

→ Therapeutic vaccine NexVax2

Proof of Concept: induction of tolerance in DQ2 TCR tg mouse



Induction → **Maintenance**

Induction	Maintenance
Daily, 3x/weekly	Weekly
Dose escalation or linear	Maintenance dose
Achieve maintenance dose	~ED20 dose

Collect spleen and analyse T cell response to peptide

- Anergy
- Treg induction – cell markers and function
- Suppression interferon-γ, IL-2
- Induction IL-10

Courtesy of Bob Anderson

PHASE 1: NEXVAX2 IN HLA DQ2.5 CELIAC DISEASE

Courtesy of Bob Anderson

Study design

- N=34 healthy HLA DQ2+(DQ8-) adults with celiac disease on gluten free diet (GFD)
- Sequentially randomized to receive 9µg (n=6), 30µg (n=6), 60µg (n=6) or 90µg (n=7) of Nexvax2® or placebo (n=9) i.d. weekly for 3 weeks.
- Double blind design
- In two dedicated GCP Phase I clinical trial centres.
- Serial interferon-gamma (IFN-γ) ELISpot assays were used to enumerate peripheral blood T-cells specific for Nexvax2® in independent lab.

Courtesy of Bob Anderson

Results: Safety/Tolerability

- Well tolerated and safe.
- Gastrointestinal adverse events more common with 60µg and 90µg of Nexvax2®
- 7/19 subjects administered 30µg, 60µg or 90µg of Nexvax2® vs 0/9 on placebo reported nausea, vomiting or diarrhoea
- 2 subjects were administered anti-emetics and two vomited (at approximately 2h or 5.5h after the initial dose).
- One subject in the 90µg cohort withdrew due to gastrointestinal symptoms graded severe.

Courtesy of Bob Anderson

Peptide Vaccination: Questions to Be Addressed

- **Role of innate immunity eliciting peptides in CD lesion**
- **Role of HLA-Class I restricted T-cell epitope(s)**
- **Repertoire of active peptides in different celiac population?**
- **Repertoire of active peptides in childhood and latent CD?**

Glutenase AN-PEP
as future therapy?
In vivo Digestion of gluten toxic peptides
Use of prolyl endoprotease from Aspergyllus Niger

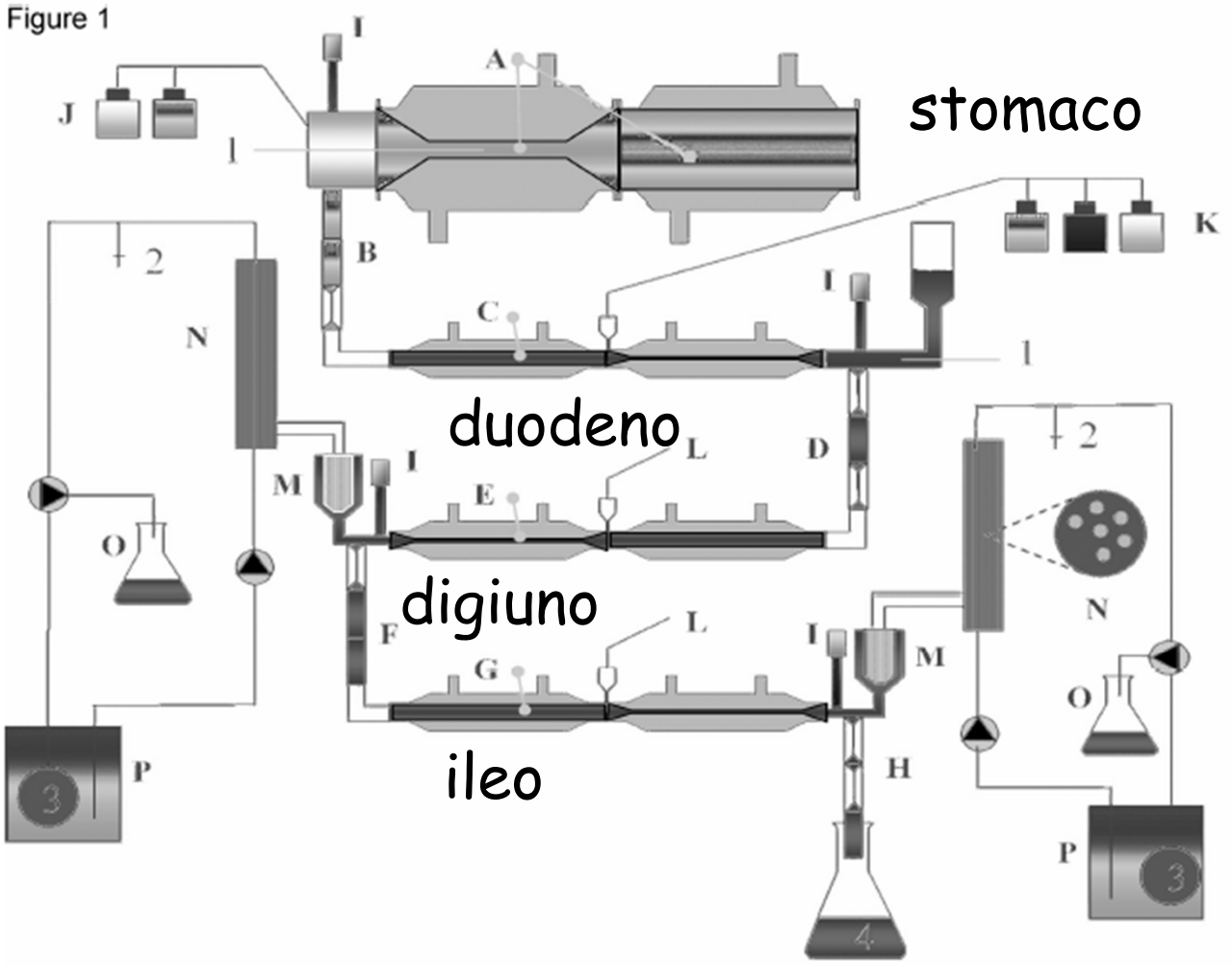
Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for celiac disease

Cristina Mitea[1], Robert Havenaar[2], Jan Wouter Drijfhout[1], Luppo Edens[3], Liesbeth Dekking[1] and Frits Koning[1]

From the [1]Dept of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands, [2]TNO Quality of Life, Zeist, The Netherlands, [3]DSM Food Specialties, Delft, The Netherlands

GUT 2008

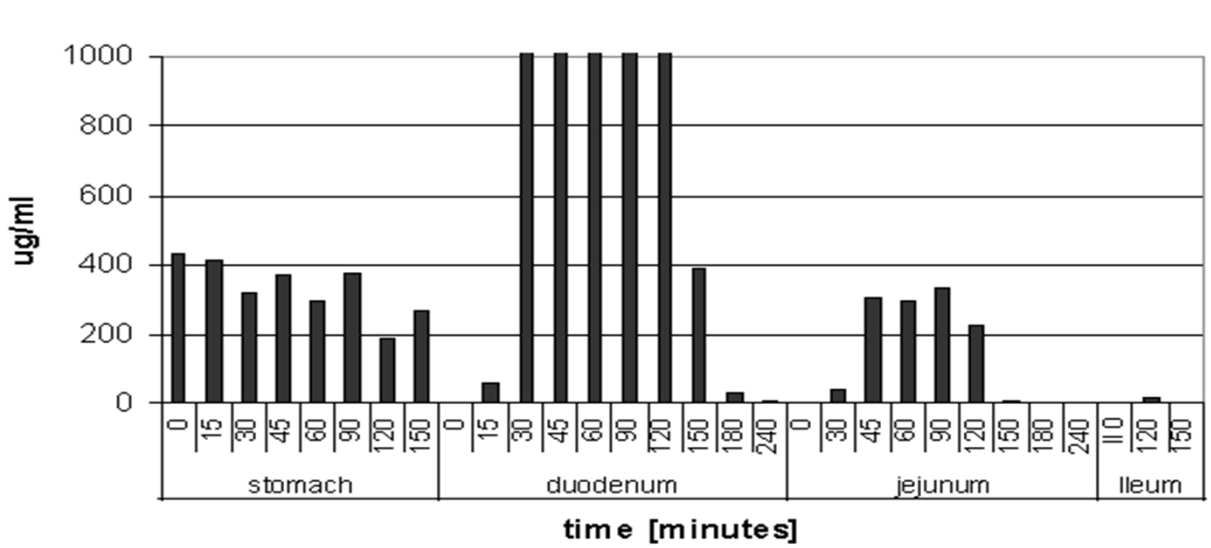
Tratto gastro intestinale artificiale



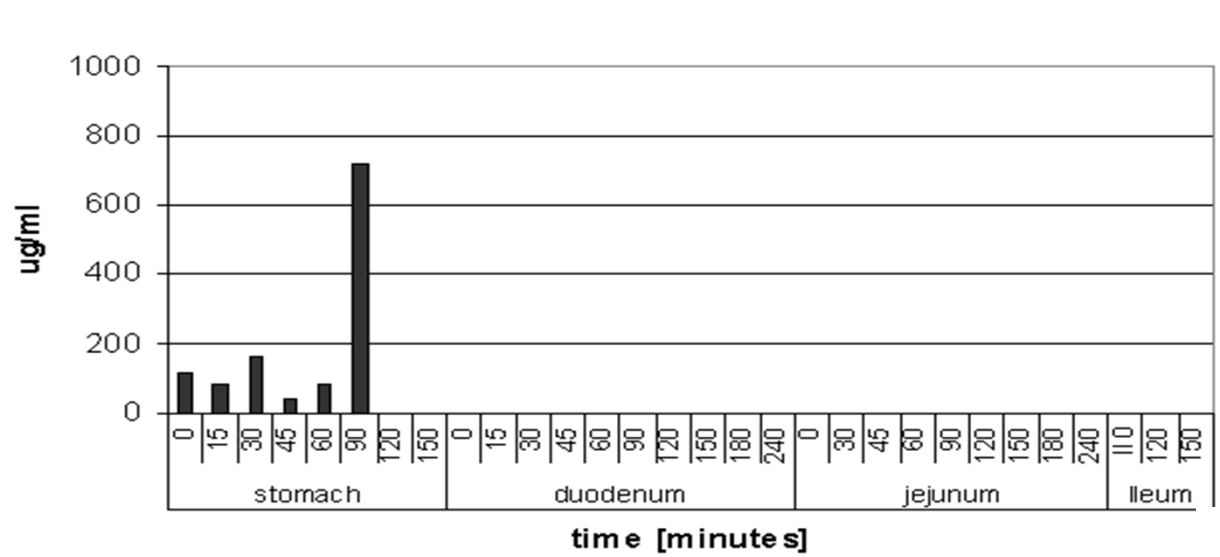
AN-PEP: a prolyl endoprotease

- Completely cut of gluten peptides in the artificial stomach
- Food grade
- Mass production in large scale fermentors

AN-PEP degrades gluten peptides



NO AN-PEP



AN-PEP

AN-PEP therapy is on Phase I clinical trial

Study design

- CD donors eat for 2 weeks glutenase with gluten or placebo
- Two weeks of gluten wash out
- Two weeks of glutenase with gluten or placebo
- Evaluation of intestinal lesion by endoscopy

Results

- No clear differences in symptoms
- No clear differences in Marsh score
- No clear differences in antibody titers

Frits Koning communication Florence 2012

AN-PEP therapy is on Phase I clinical trial

The second clinical trial is ongoing

12 healthy donors eat 300 ml of meal, and a sample of “gastro-enteric” digested meal is taken at some time points to evaluate the digestion of gluten by ELISA and western blot.

The study is on progress.

Istituto di Scienze dell'Alimentazione,
CNR Avellino

Prospettive di terapia
Conclusioni

1. Uso delle cellule T regolatorie: proof of concept

2. Allo studio clinico il vaccino desensibilizzante a base dei peptidi del glutine immunodominanti:

3. Allo studio clinico la pillola a base di enzimi che digeriscono il glutine a livello gastrico

Applicabilità? Terapia a lungo termine o per consumi sporadici o accidentali di glutine?

Quali sono le quantità di glutine permesse?

Carmen Gianfrani

Salvatore Auricchio
Riccardo Troncone

Maria Grazia Roncarolo

Bob Anderson