PathWest
LABORATORY MEDICINE WA

Princess Margaret Hospital for Children

SIR CHARLES GAIRDNER HOSPITAL

SOME IMMUNOLOGICAL ASPECTS…

Australasian Gastrointestinal Pathology Society AGM
28 oct 2016

Andrew McLean-Tooke
SCGH, Princess Margaret Hospitals and PathWest

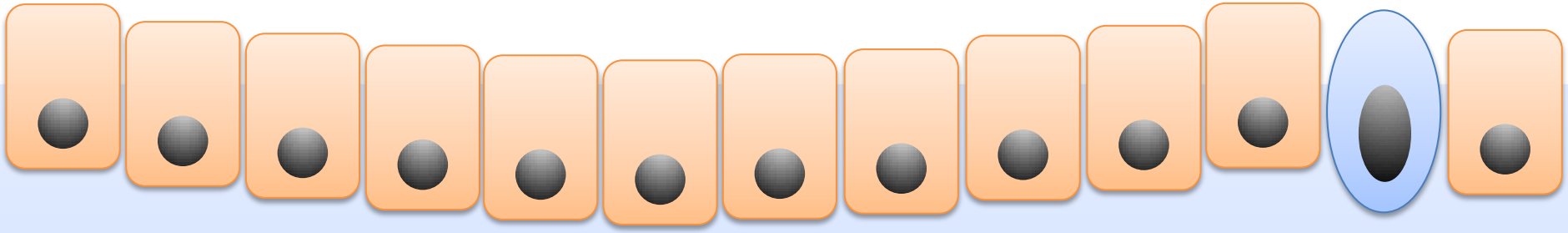
Wheat and gluten

- Gluten is a protein mixture found in wheat
- Complex mixture
  - α/β-, γ- and ω-gliadins
  - HMW and LMW glutenins
- Relatively resistant to digestive enzymes

So what happens in CD?

- CD due to immune response to gluten
- Environmental and genetic risk factors
- Activation of immune cells in small bowel
- Malabsorption results in clinical symptoms
- Only known treatment is gluten avoidance

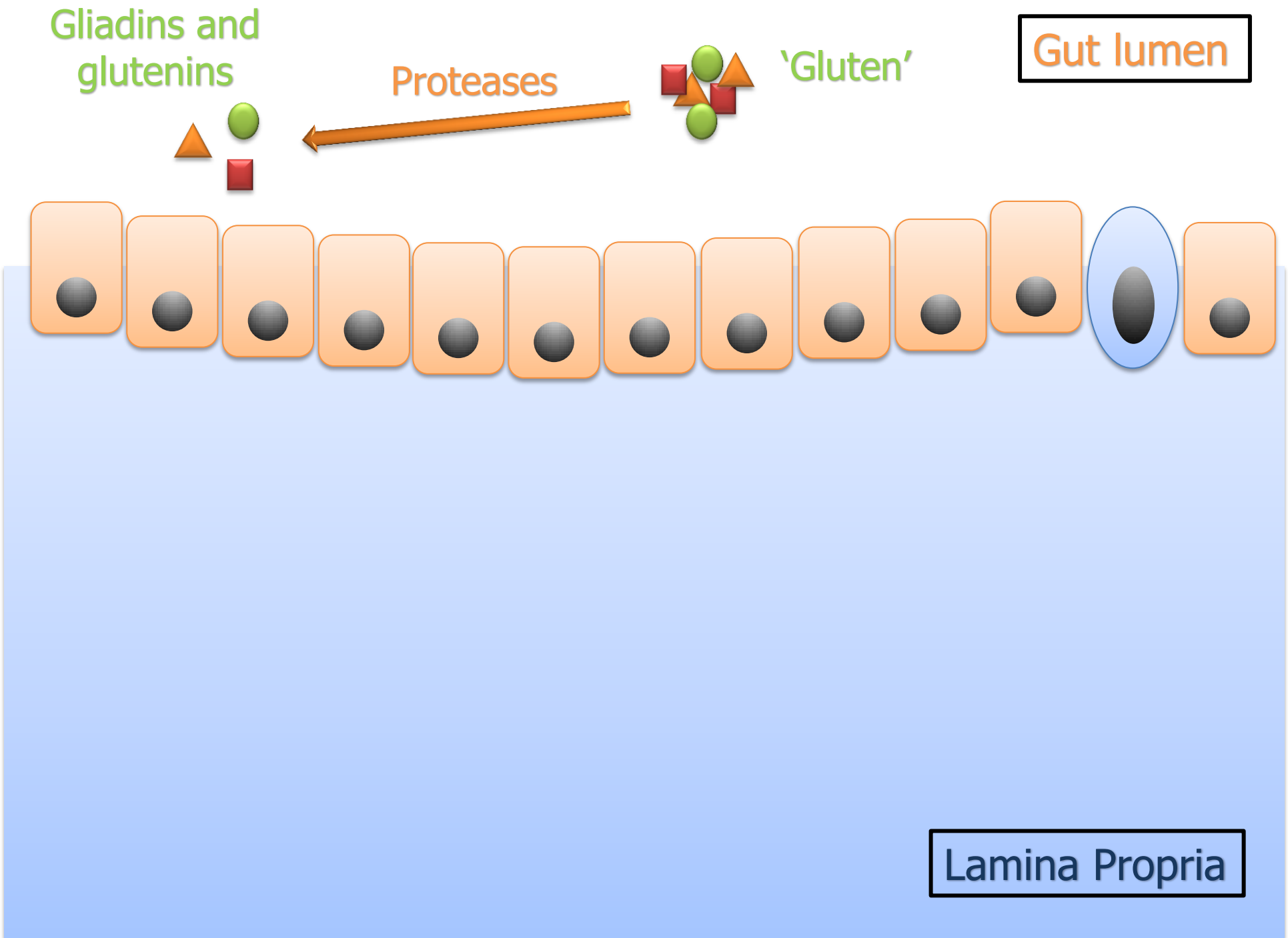
Gut lumen

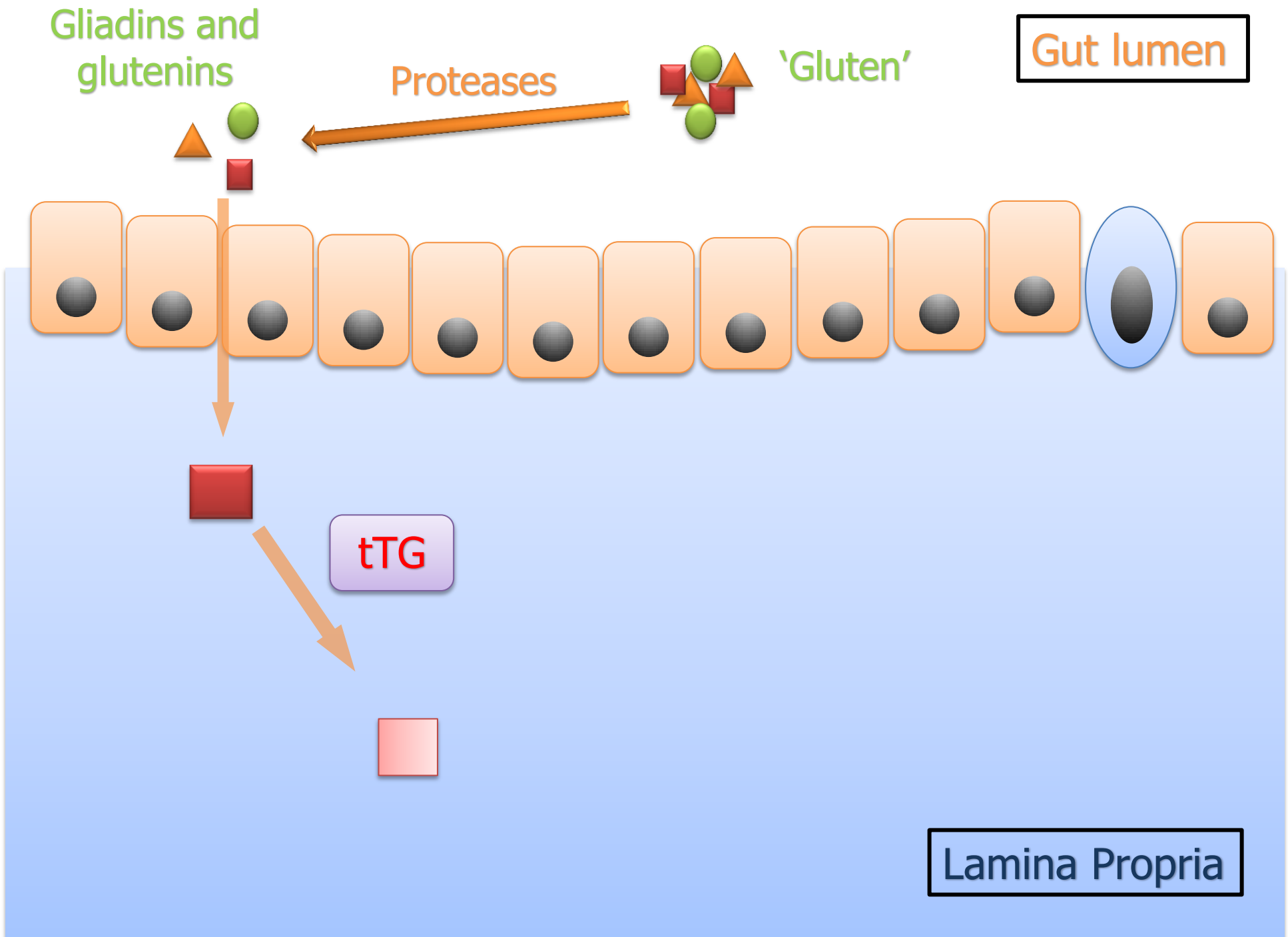


Enterocytes

IELs

Lamina Propria


Gliadins and glutenins
Proteases
'Gluten'
Gut lumen
Lamina Propria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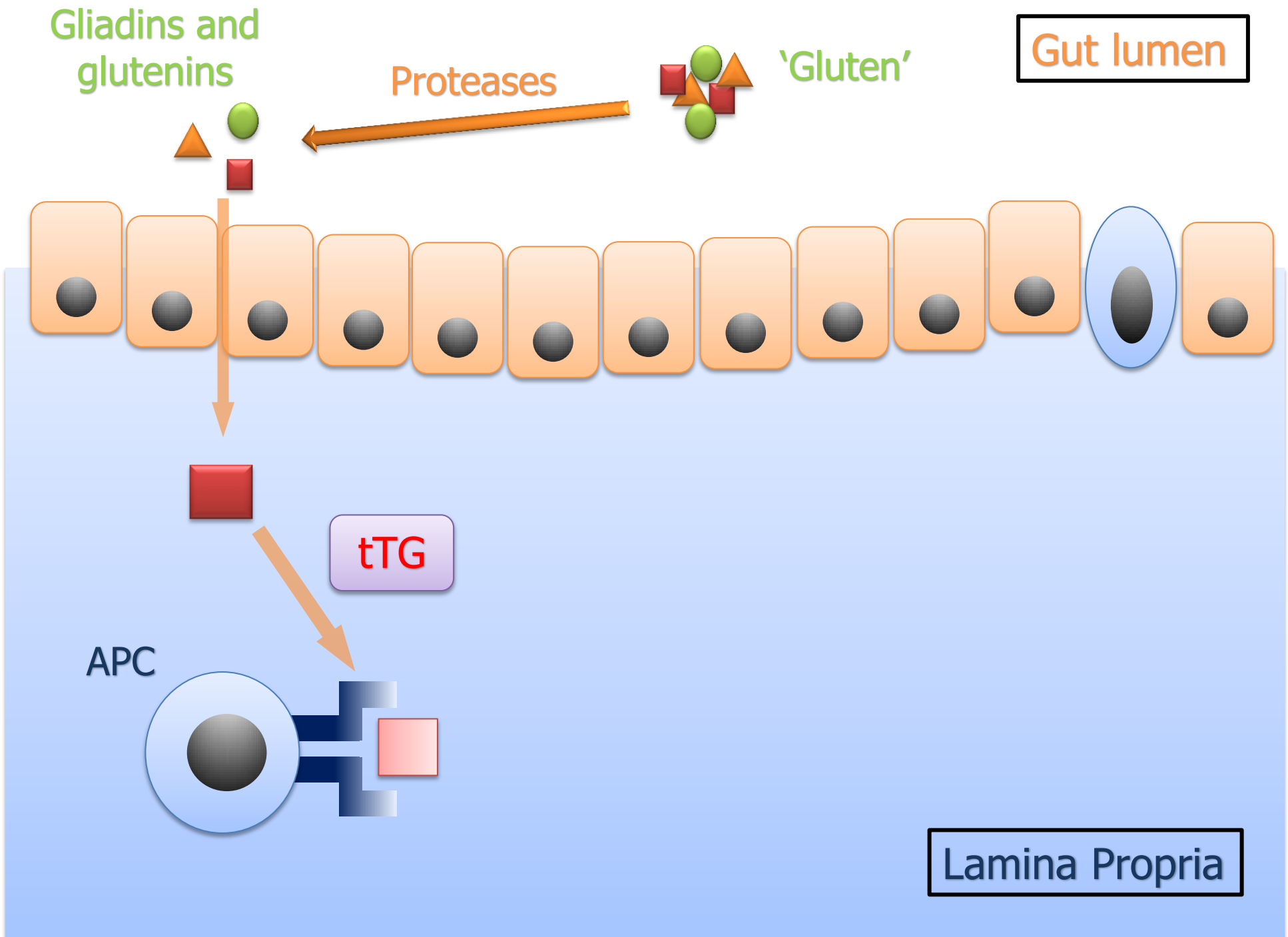
Gliadins and glutenins
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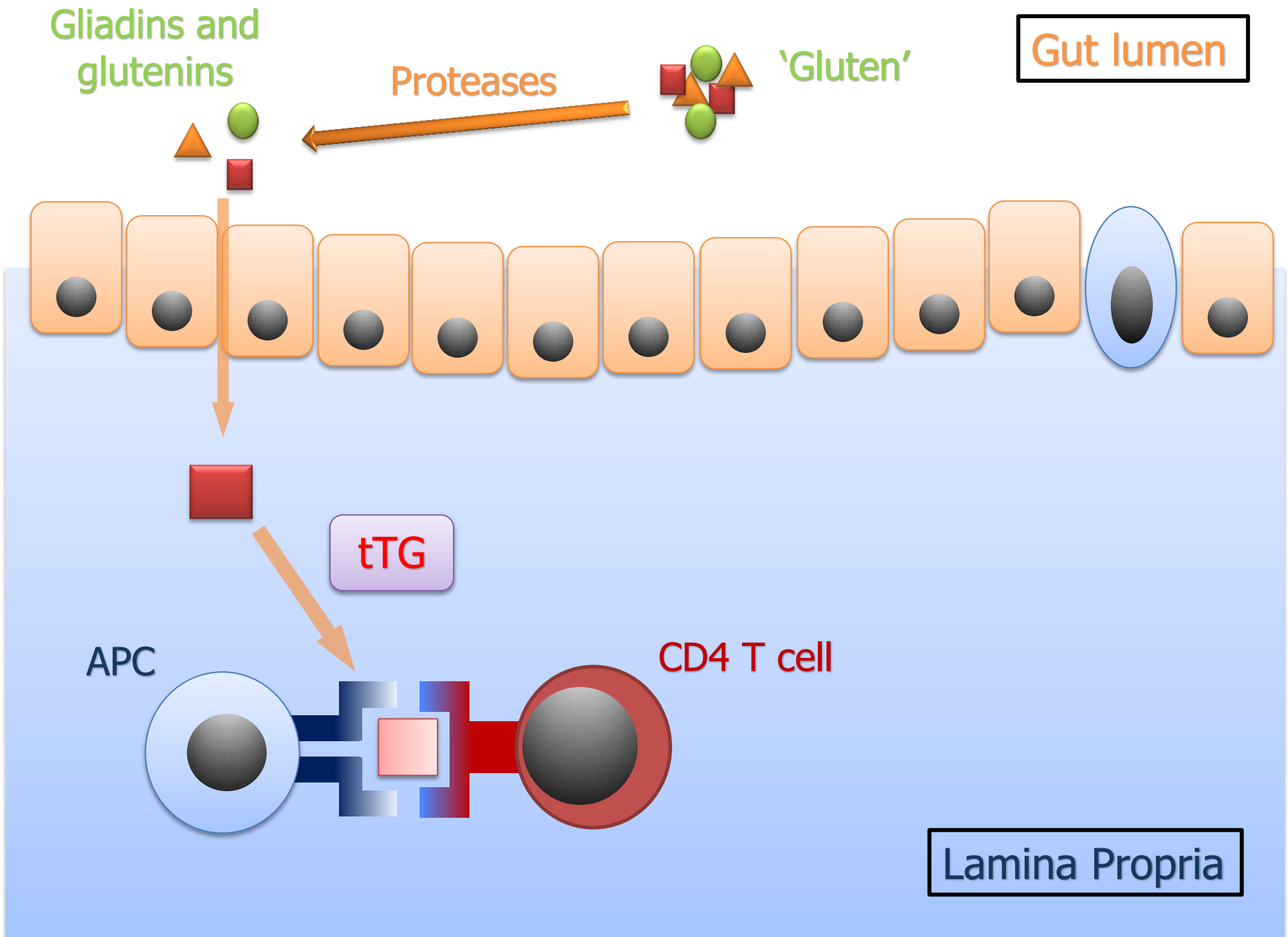
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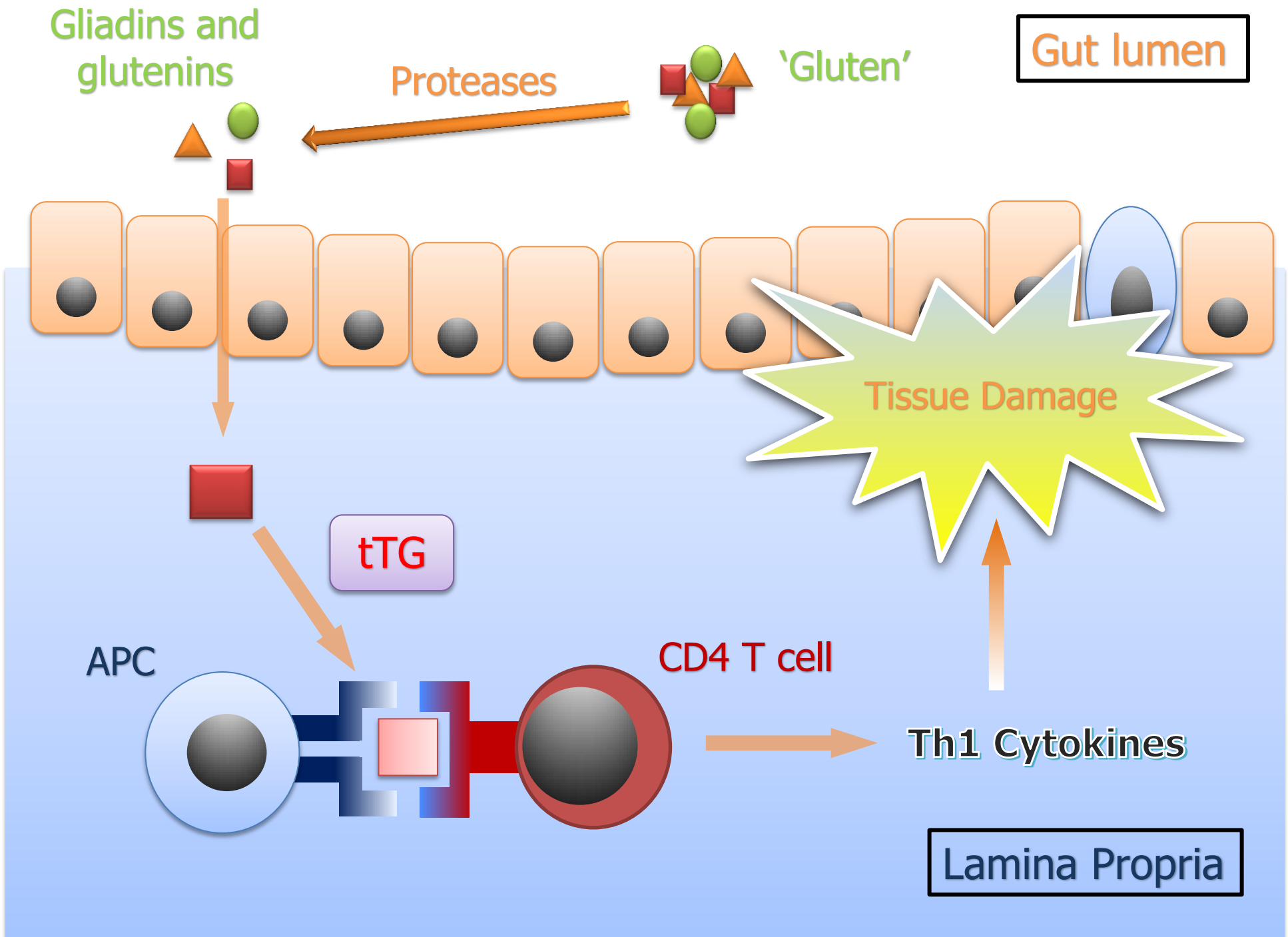
Gliadins and glutenins
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Th1 Cytokines
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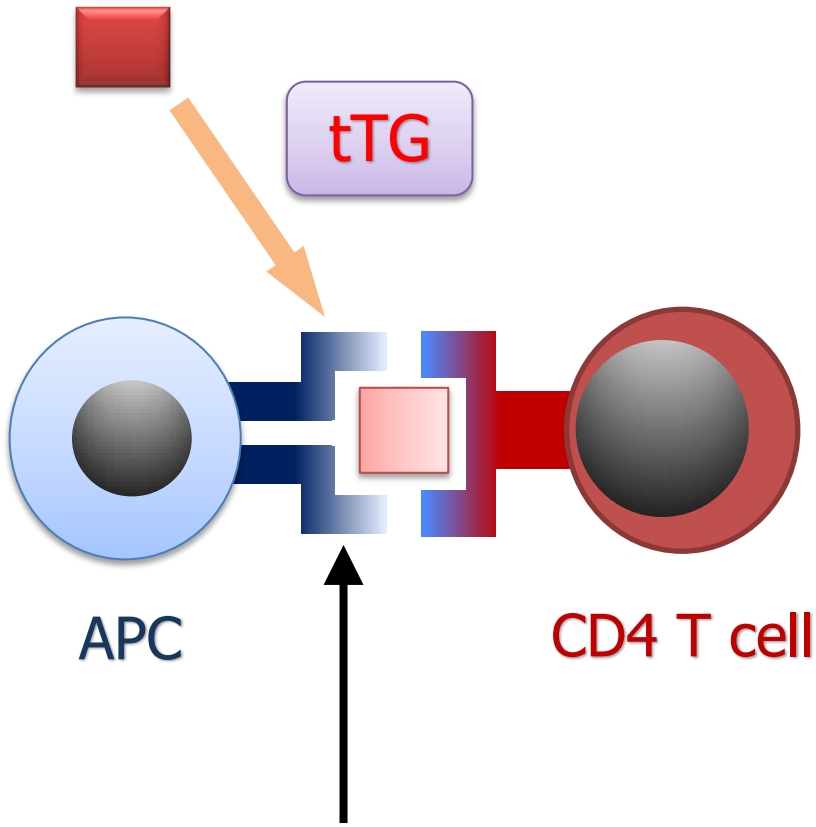
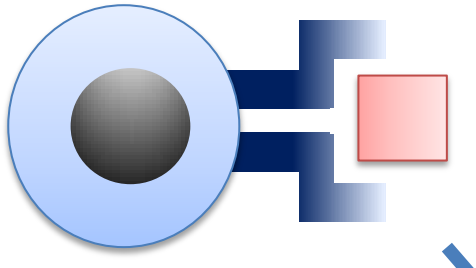
Role of HLA

- HLA-DQ molecules are critical in developing CD
- Certain HLA-DQ genetic variants almost invariably present
- These variants critical for response to gluten
- At least 41 non-HLA loci contribute to risk

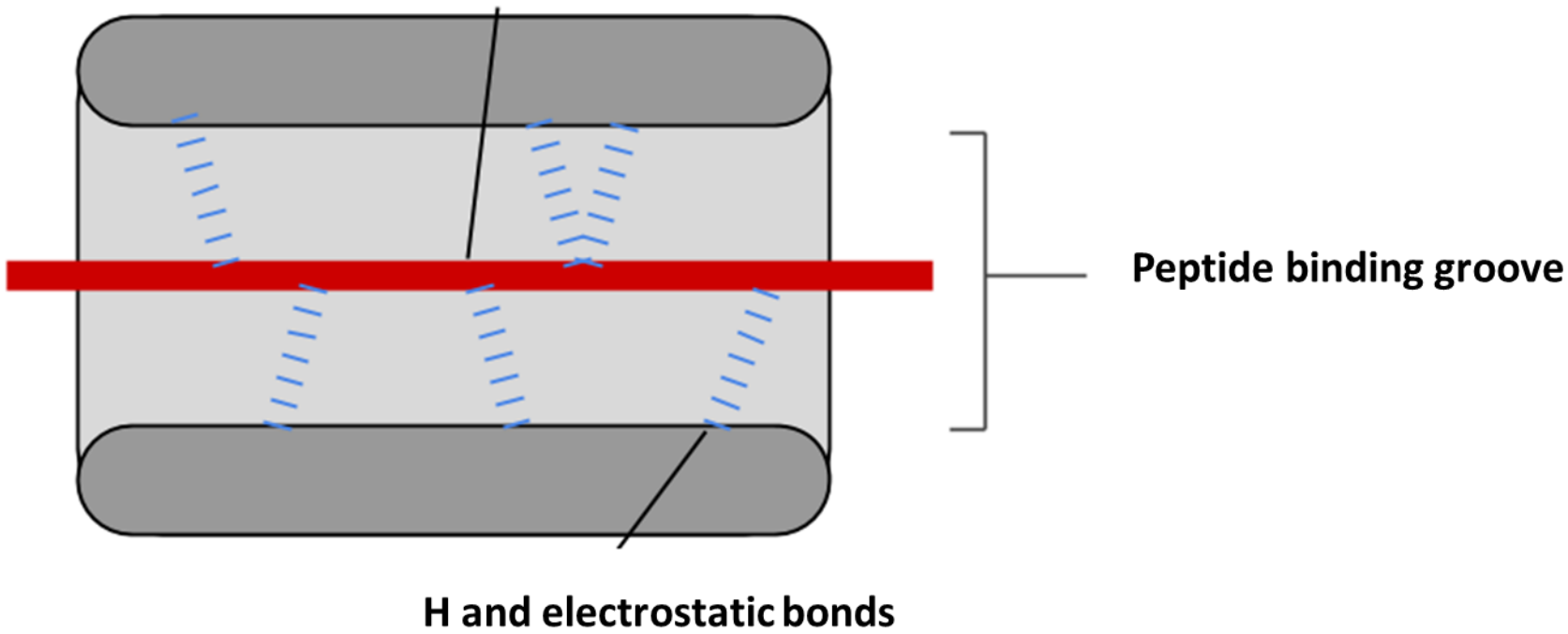


HLA molecule capable of recognising gluten peptides



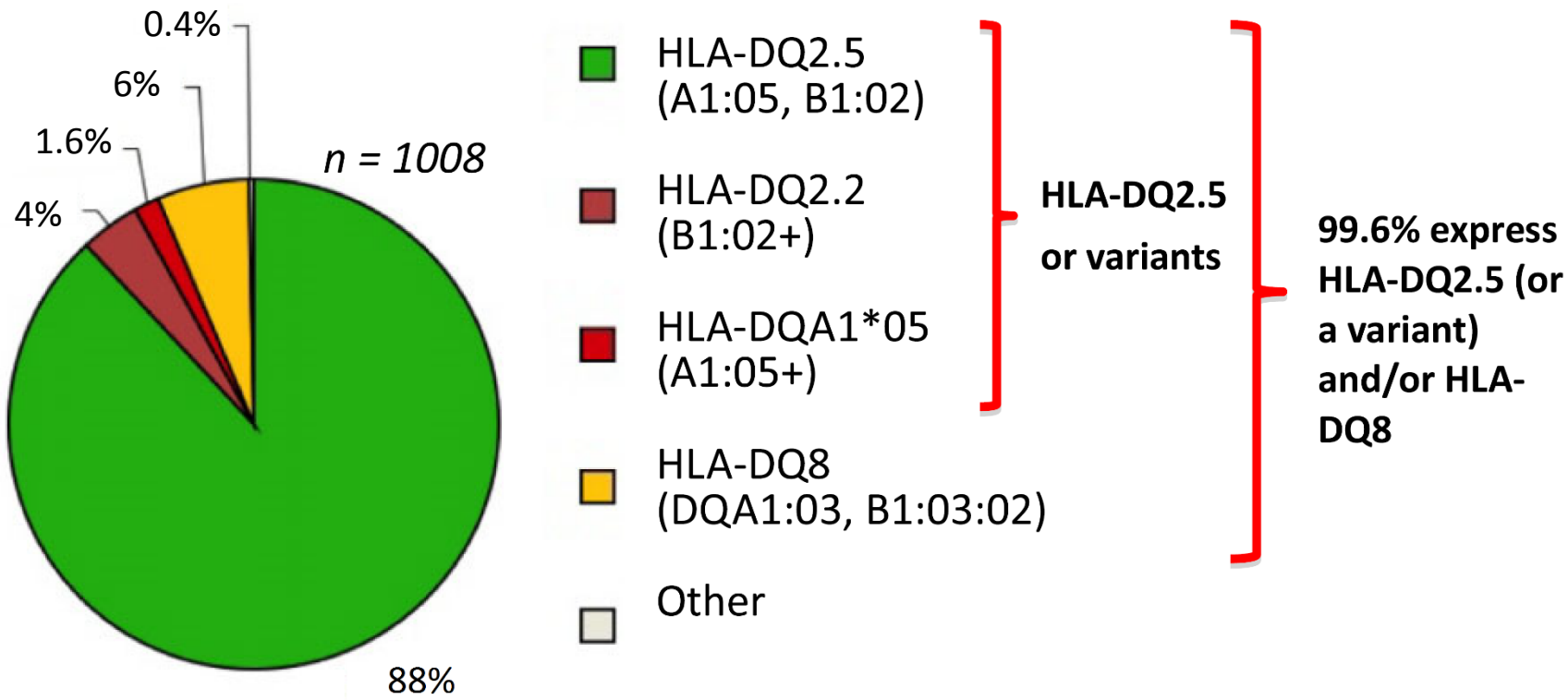
APC

MHC Class II



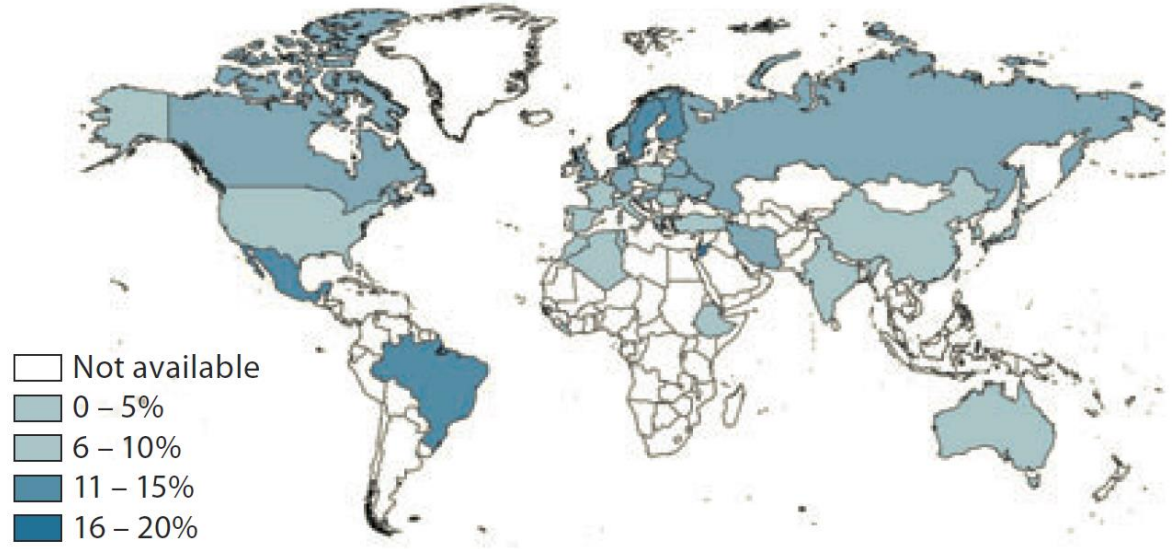
Risk category	HLA genotypes	Absolute HLA risk (%)
Low risk	DQ7/DQ7	0.0000
	DQX/DQX	0.0433
	DQ7/DQX	0.0470
Intermediate risk	DQ2.2/DQX	0.1661
	DQ8/DQ7	0.2765
	DQ8/DQX	0.5326
	DQ2.5/DQ8	1.5769
	DQ2.2/DQ2.2	1.6366
	DQ8/DQ8	1.6366
	DQ2.5/DQ7	2.2587
	DQ2.5/DQX	2.6194
	DQ8/DQ2.2	2.9600
	DQ2.2/DQ7	3.7232
High risk	DQ2.5/DQ2.2	7.7079
	DQ2.5/DQ2.5	12.8137

Romanos *et al.* Ann Gastro Hepatol. 2010;1:11-18



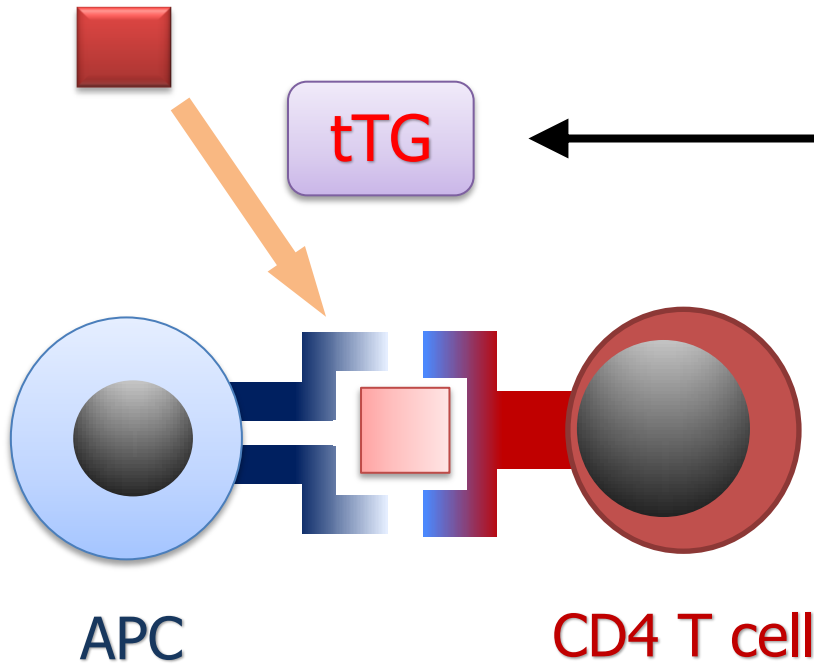
Tye-Din *et al.* Int Med J 2015;45:441

- Frequency of HLA-DQ2 haplotype



- Frequency of HLA-DQ8 haplotype

Abadie *et al.* Ann Rev Imm 2011;29:493



Modification of gluten peptides

Tissue Transglutaminase (tTG)

- Ubiquitous enzyme
- Cross links peptides
- Involved in tissue repair, ECM stabilisation, cell adhesion
- Also catalyses other reactions including deamidation

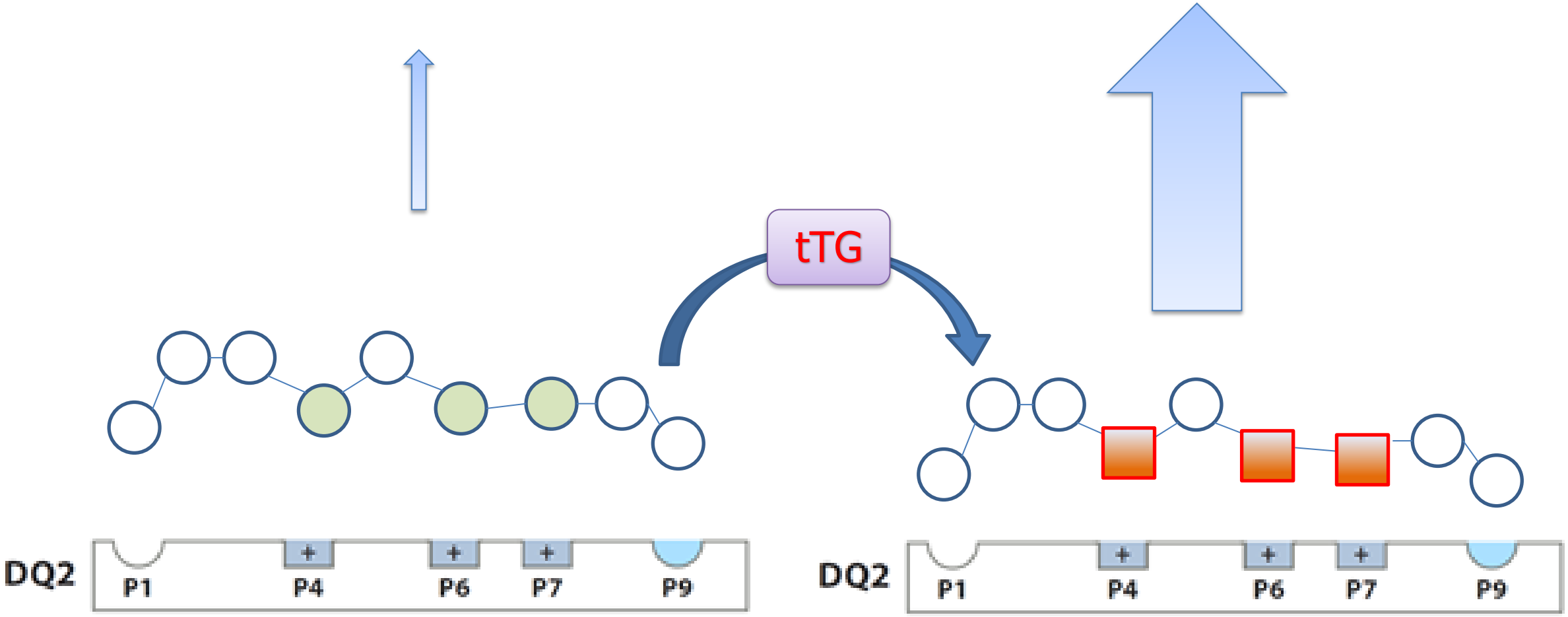
L-Glutamine

Glutamic Acid

Walker-Smith *et al.* Arch Dis Childhood 1990;65:99

T cell receptor signal

T cell receptor signal



T cell receptor signal

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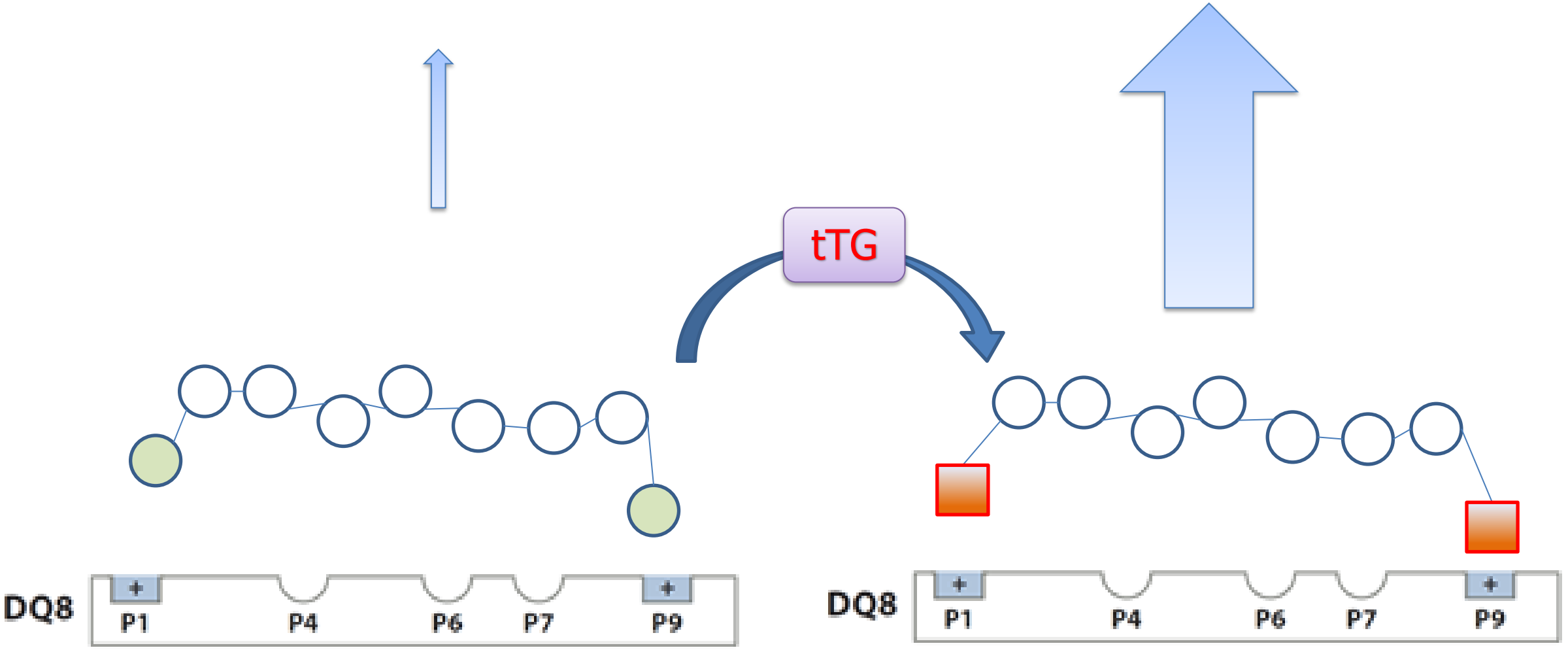
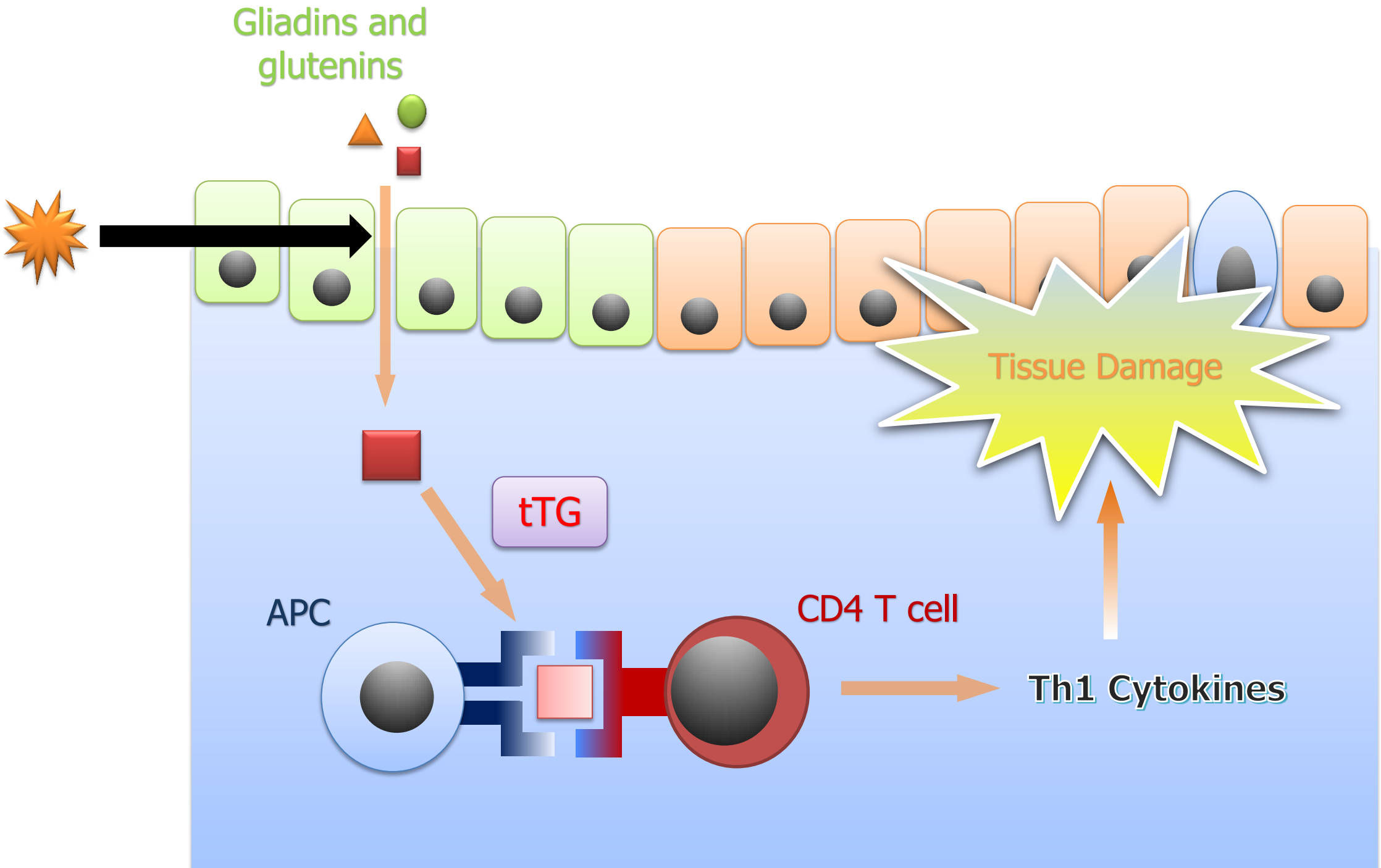


EPITOPE	PEPTIDE-BINDING REGISTERY									EPITOPE	PEPTIDE-BINDING REGISTERY								
	1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9
DQ2.5 restricted epitopes																			
DQ2.5-glia-α1a	P	F	P	Q	P	**E**	L	P	Y	DQ2.5-hor-2	P	Q	P	**E**	Q	P	F	P	Q
DQ2.5-glia-α1b	P	Y	P	Q	P	**E**	L	P	Y	DQ2.5-hor-3	P	I	P	**E**	Q	P	Q	P	Y
DQ2.5-glia-α2	P	Q	P	**E**	L	P	Y	P	Q	DQ2.5-sec-1	P	F	P	Q	P	**E**	Q	P	F
DQ2.5-glia-α3	F	R	P	**E**	Q	P	Y	P	Q	DQ2.5-sec-2	P	Q	P	**E**	Q	P	F	P	Q
DQ2.5-glia-γ1	P	Q	Q	S	F	P	**E**	Q	Q	DQ2.5-ave-1a	P	Y	P	E	Q	**E**	E	P	F
DQ2.5-glia-γ2	I	Q	P	**E**	Q	P	A	Q	L	DQ2.5-ave-1b	P	Y	P	E	Q	**E**	Q	P	F
DQ2.5-glia-γ3	Q	Q	P	**E**	Q	P	Y	P	Q	**DQ2.2 restricted epitopes**									
DQ2.5-glia-γ4a	S	Q	P	**E**	Q	**E**	F	P	Q	DQ2.2-glut-L1	P	F	S	**E**	Q	**E**	Q	P	V
DQ2.5-glia-γ4b	P	Q	P	**E**	Q	**E**	F	P	Q	**DQ8 restricted epitopes**									
DQ2.5-glia-γ4c	Q	Q	P	**E**	Q	P	F	P	Q	DQ8-glia-α1	**E**	G	S	F	Q	P	S	Q	**E**
DQ2.5-glia-γ4d	P	Q	P	**E**	Q	P	F	C	Q	DQ8-glia-γ1a	**E**	Q	P	Q	Q	P	F	P	Q
DQ2.5-glia-γ5	Q	Q	P	F	P	**E**	Q	P	Q	DQ8-glia-γ1b	**E**	Q	P	Q	Q	P	Y	P	**E**
DQ2.5-glia-ω1	P	F	P	Q	P	**E**	Q	P	F	DQ8-glut-H1	Q	G	Y	Y	P	T	S	P	Q
DQ2.5-glia-ω2	P	Q	P	**E**	Q	P	F	P	W	**DQ8.5 restricted epitopes**									
DQ2.5-glut-L1	P	F	S	**E**	Q	**E**	Q	P	V	DQ8.5-glia-α1	**E**	G	S	F	Q	P	A	Q	**E**
DQ2.5-glut-L2	F	S	Q	Q	Q	**E**	S	P	F	DQ8.5-glia-γ1	P	Q	Q	S	F	P	**E**	Q	**E**
DQ2.5-hor-1	P	F	P	Q	P	**E**	Q	P	F	DQ8.5-glut-H1	Q	G	Y	Y	P	T	S	P	Q

Sollid *et al.* Immunogenetics 2012;64:455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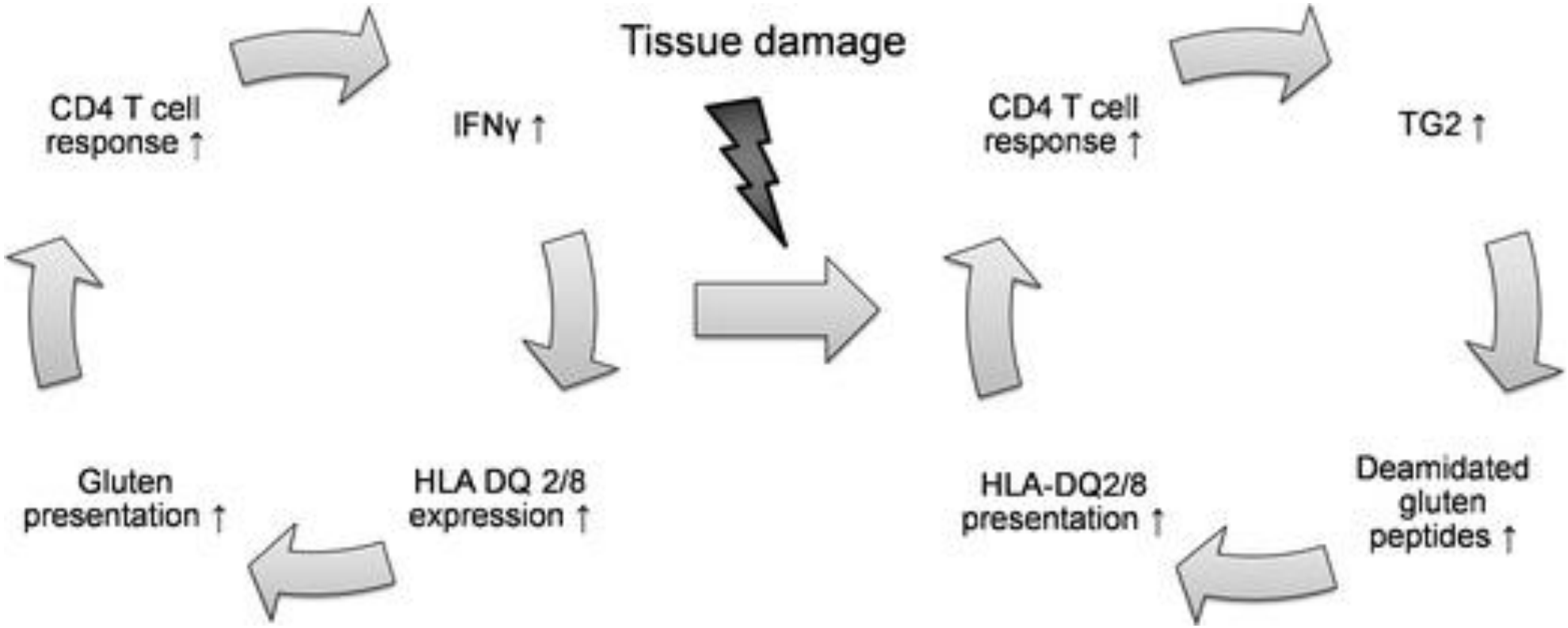
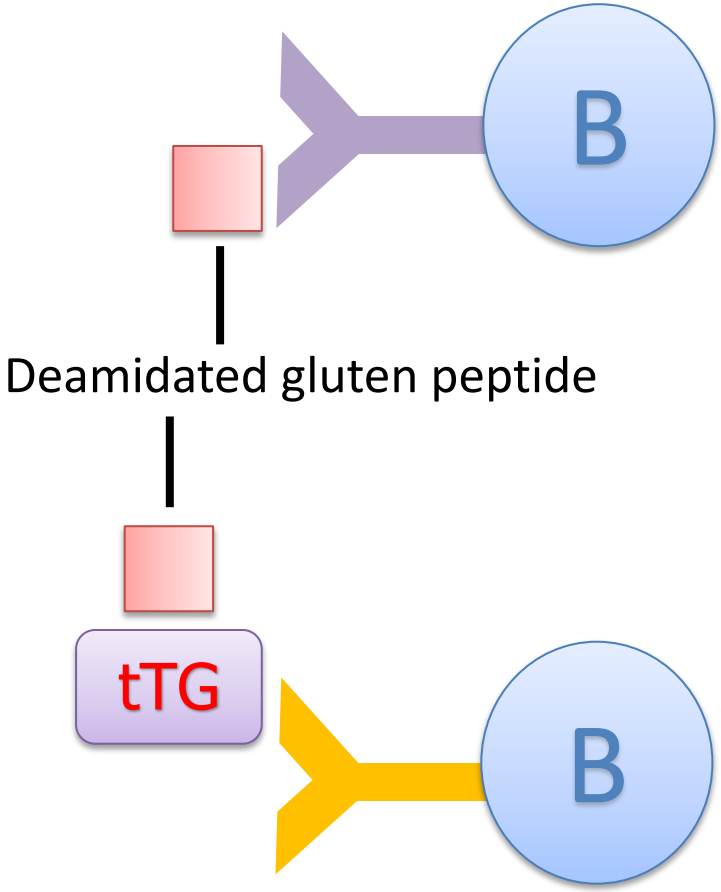


Tjon *et al.* Immunogenetics. 2010; 62:641

Serological diagnosis of CD

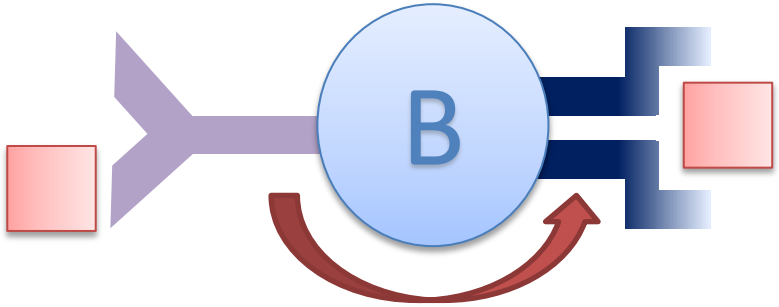
- Serological assessment
  - Anti-EMA antibodies
  - Anti-tTG antibodies
  - Anti-DGP antibodies

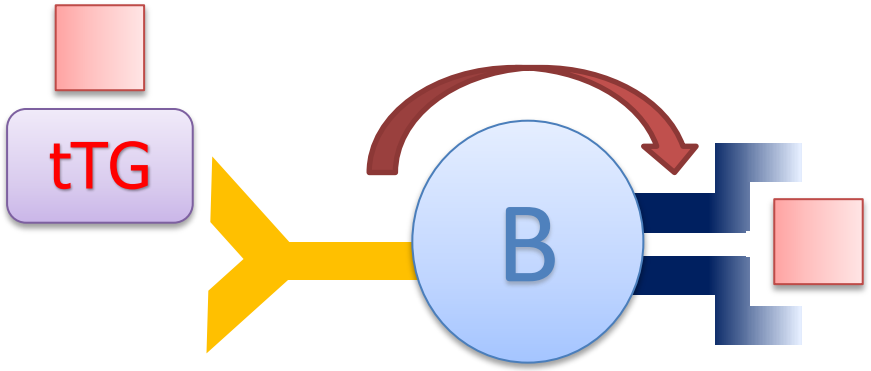
DGP specific B cell



TTG specific B cell

DGP specific B cell

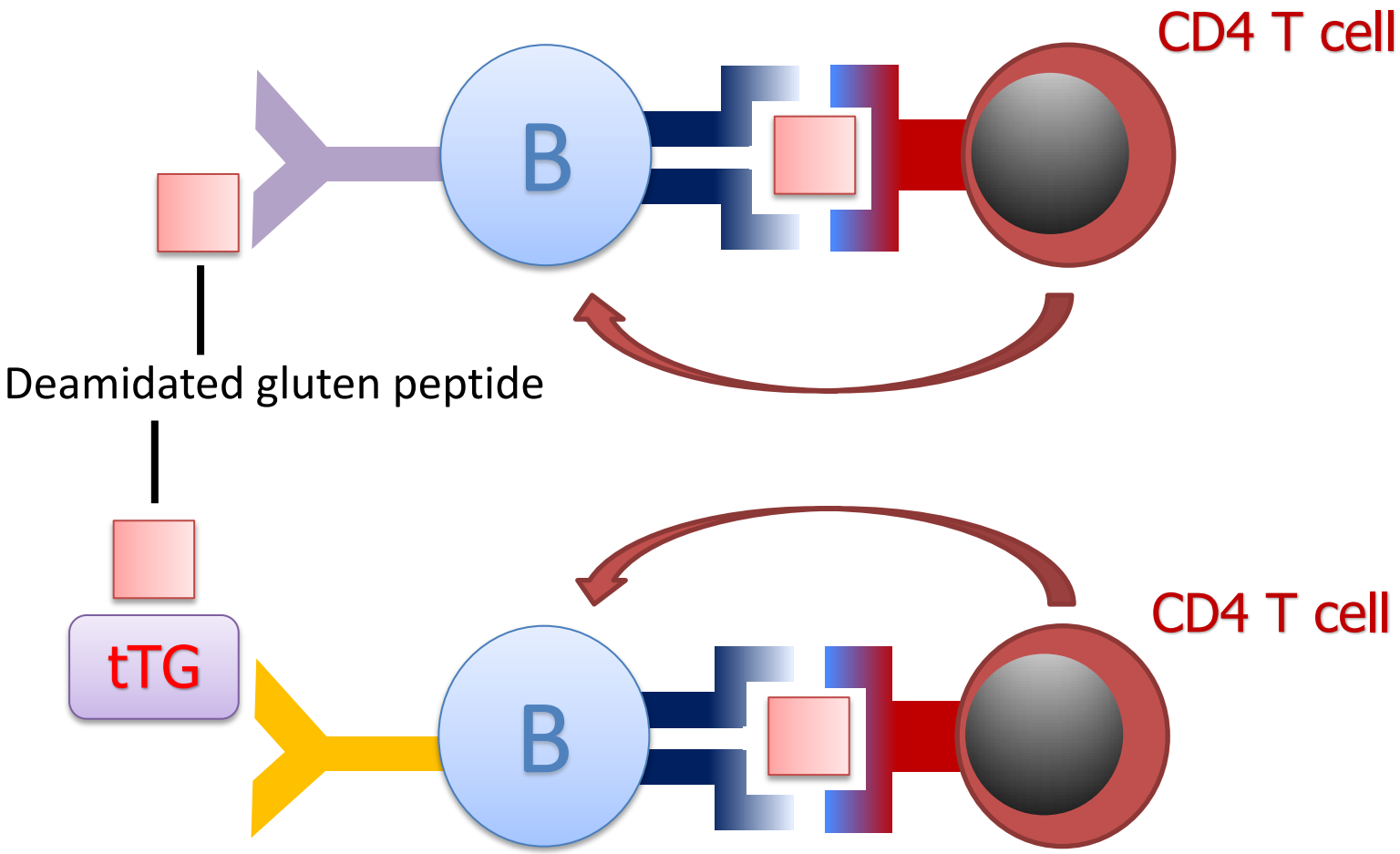




TTG specific B cell

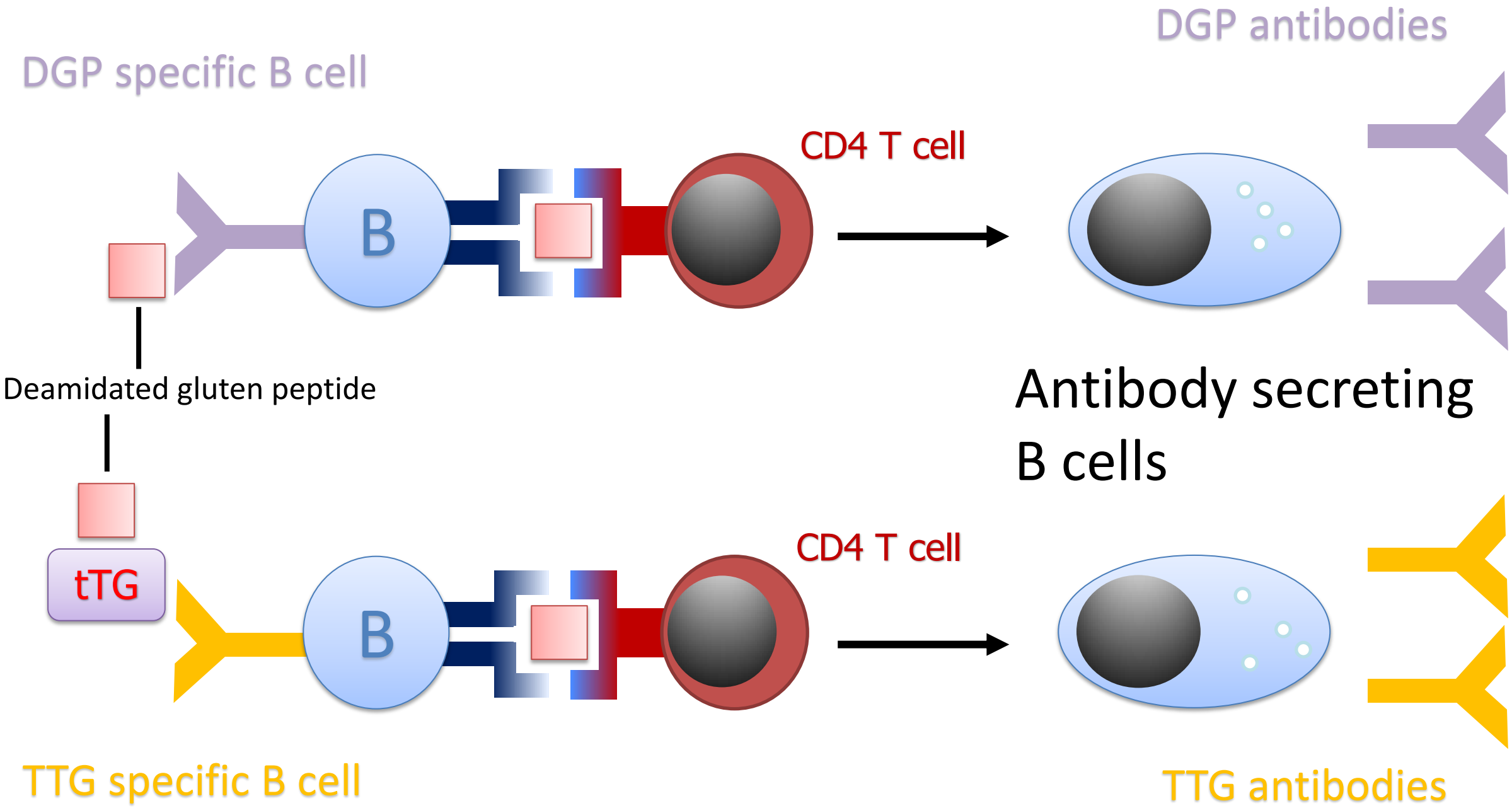
- Immunoglobulin/antigen complex internalised and broken down
- Peptides presented on surface with HLA Class II

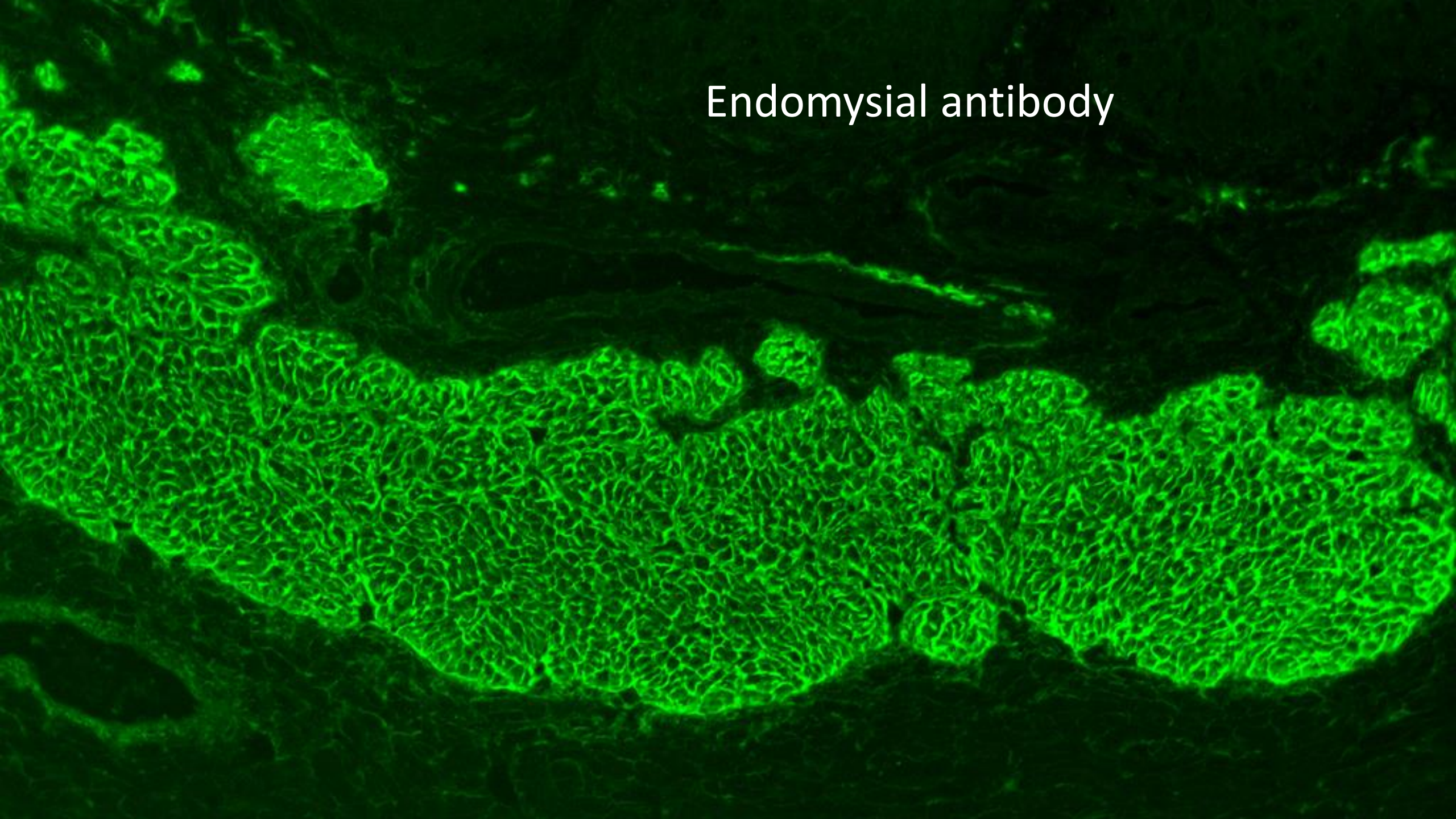
DGP specific B cell



TTG specific B cell

- Gluten specific T cells “see” the gluten peptides
- Provide ‘help’ to B cells activating them


DGP antibodies
DGP specific B cell
CD4 T cell
B
Deamidated gluten peptide
Antibody secreting
B cells
tTG
CD4 T cell
B
TTG specific B cell
TTG antibodies


Endomysial antibody

TTG antibodies

- Identified as major target of EMA antibodies
- Allowed development of ELISA based systems
- IgA tTG antibodies mainstay of many algorithms

DGP antibodies

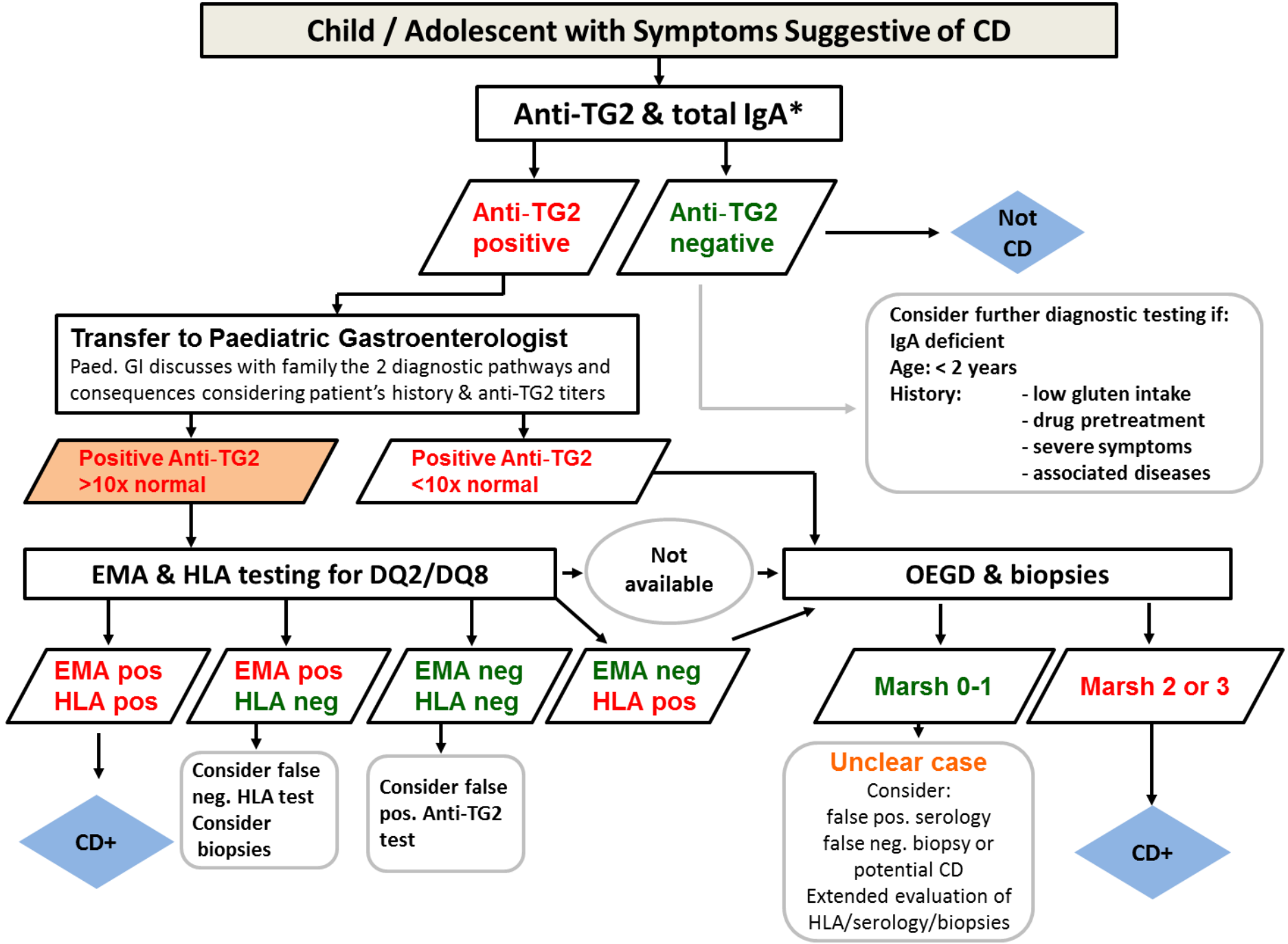
- Gliadin peptides antibodies poor specificity
- Abs to deamidated gliadin peptides a higher specificity systems
- IgG to DGP better than IgG to tTG
- Benefit in children <2yrs

Revised ESPGHAN Guidelines 2012

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease

*S. Husby, †S. Koletzko, ‡I.R. Korponay-Szabó, §M.L. Mearin, ||A. Phillips, ¶R. Shamir, #R. Troncone, **K. Giersiepen, ††D. Branski, ‡‡C. Catassi, §§M. Lelgeman, ||||M. Mäki, ¶¶C. Ribes-Koninckx, ##A. Ventura, and ****K.P. Zimmer, for the ESPGHAN Working Group on Coeliac Disease Diagnosis, on behalf of the ESPGHAN Gastroenterology Committee

Journal of Pediatric Gastroenterology and Nutrition 2012;54: 136–160


Child / Adolescent with Symptoms Suggestive of CD
Anti-TG2 & total IgA*
Anti-TG2 positive
Anti-TG2 negative
Not CD
Consider further diagnostic testing if:
IgA deficient
Age: < 2 years
History:
- low gluten intake
- drug pretreatment
- severe symptoms
- associated diseases
Transfer to Paediatric Gastroenterologist
Paed. GI discusses with family the 2 diagnostic pathways and consequences considering patient's history & anti-TG2 titers
Positive Anti-TG2 >10x normal
Positive Anti-TG2 <10x normal
EMA & HLA testing for DQ2/DQ8
Not available
OEGD & biopsies
EMA pos HLA pos
EMA pos HLA neg
EMA neg HLA neg
EMA neg HLA pos
Marsh 0-1
Marsh 2 or 3
CD+
Consider false neg. HLA test Consider biopsies
Consider false pos. Anti-TG2 test
Unclear case
Consider:
false pos. serology
false neg. biopsy or potential CD
Extended evaluation of HLA/serology/biopsies
CD+

AJG The American Journal of GASTROENTEROLOGY

Letter to the Editor

Am J Gastroenterol 2015; 110:1504–1505; doi:10.1038/ajg.2015.242

Should ESPGHAN Guidelines for Serologic Diagnosis of Celiac Disease be Used in Adults? A Prospective Analysis in an Adult Patient Cohort With High Pretest Probability

Emilia Sugai PhD[1], Hui J Hwang MD[1], Horacio Vázquez MD[1], María L Moreno MD[1], Florencia Costa MD[1], Gabriela Longarini MD[1], María I Pinto-Sánchez MD[1,2], Sonia Niveloni MD[1], Edgardo Smecuol MD[1], Roberto M Mazure MD[1], Elena F Verdu MD[2], Eduardo Mauriño MD[1] and Julio C Bai MD[1,3]

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[2]Farncombe Family Digestive Research Institute, McMaster University, Hamilton, Ontario, Canada
[3]Universidad del Salvador, Buenos Aires, Argentina

Correspondence: Julio C. Bai, MD, Small Bowel Section, Department of Medicine, Dr. C. Bonorino Udaondo Gastroenterology Hospital, Av. Caseros 2061, Buenos Aires (1264), Argentina. E-mail: jbai@intramed.net

The American Journal of
AP&T Alimentary Pharmacology and Therapeutics

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Correspo
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Original Scientific Paper

The presence of anti-endomysial antibodies and the level of anti-tissue transglutaminases can be used to diagnose adult coeliac disease without duodenal biopsy

R. Tortora[1,*], N. Imperatore[1], P. Capone[1], G. D. De Palma[2], G. De Stefano[1], N. Gerbino[1], N. Caporaso[1] and A. Rispo[1]

Version of Record online: 28 SEP 2014

DOI: 10.1111/apt.12970

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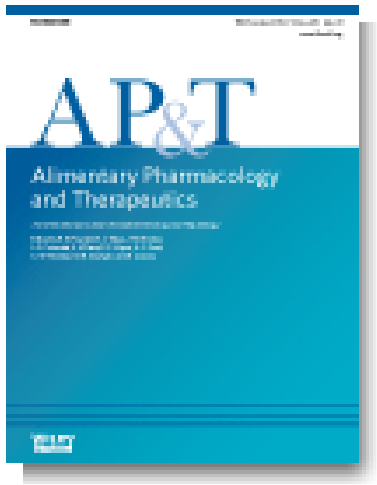

Alimentary Pharmacology & Therapeutics

Volume 40, Issue 10, pages 1223–1229, November 2014

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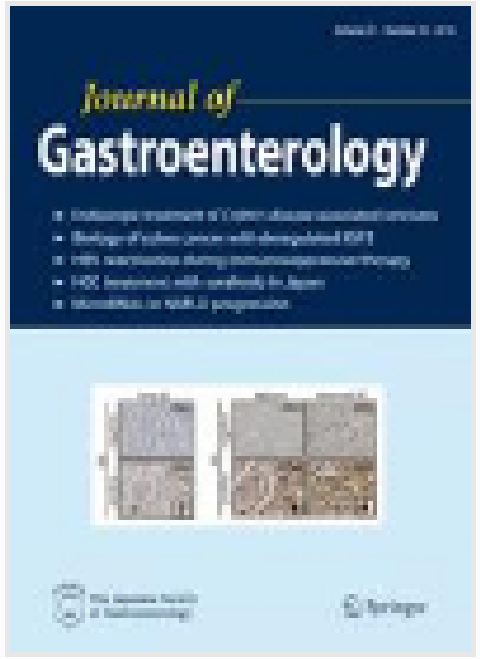

Alimentary Pharmacology

Identification of a serum transglutaminase threshold value for the noninvasive diagnosis of symptomatic adult celiac disease patients: a retrospective study

Authors | Authors and affiliations

Marco Di Tola, Mariacatia Marino, Simone Goetze, Rossella Casale, Sara Di Nardi, Raffaele Borghini, Giuseppe Donat
Antonio Tiberti, Antonio Picarelli

Original Article—Alimentary Tract

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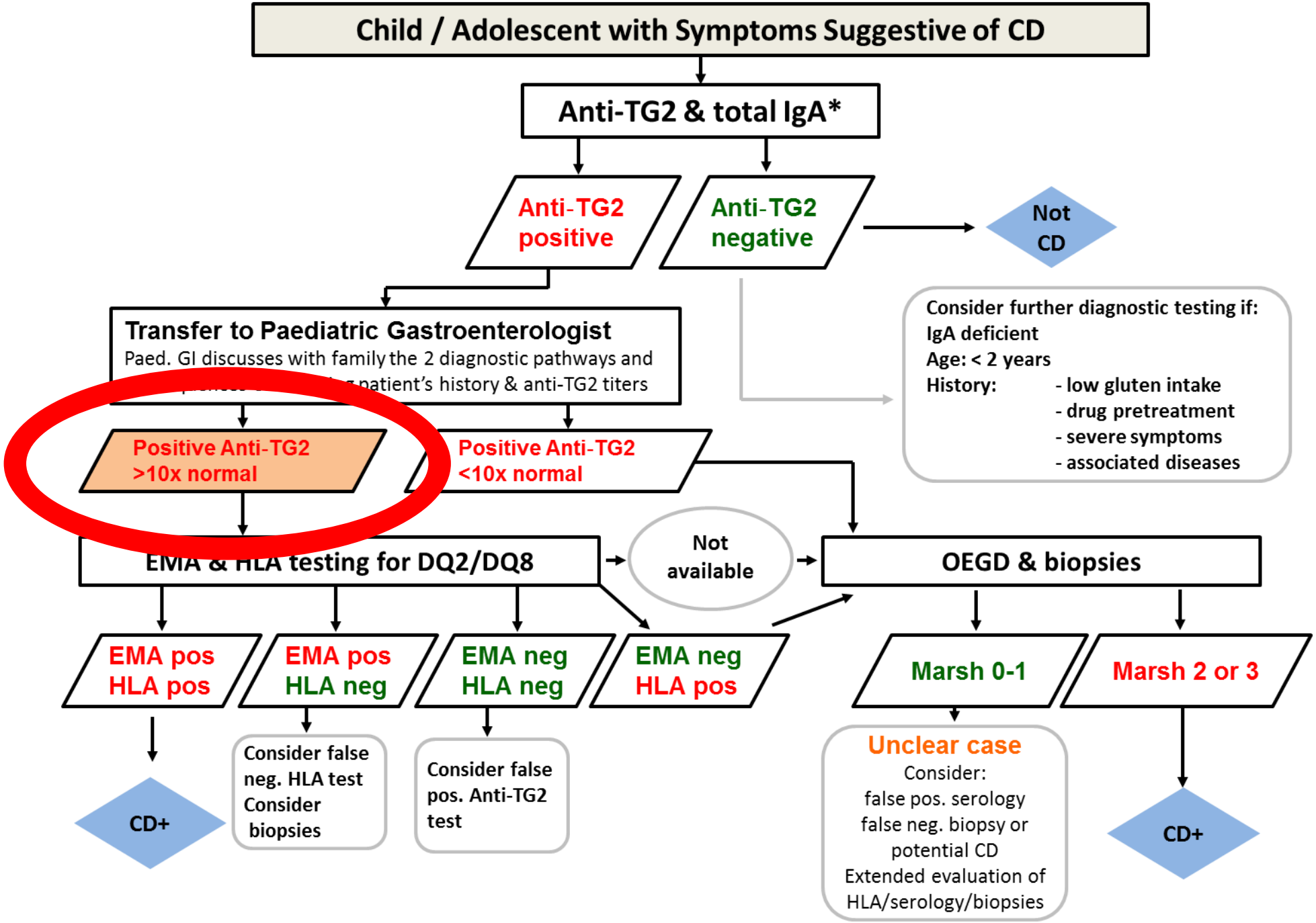
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patient's history & anti-TG2 titers
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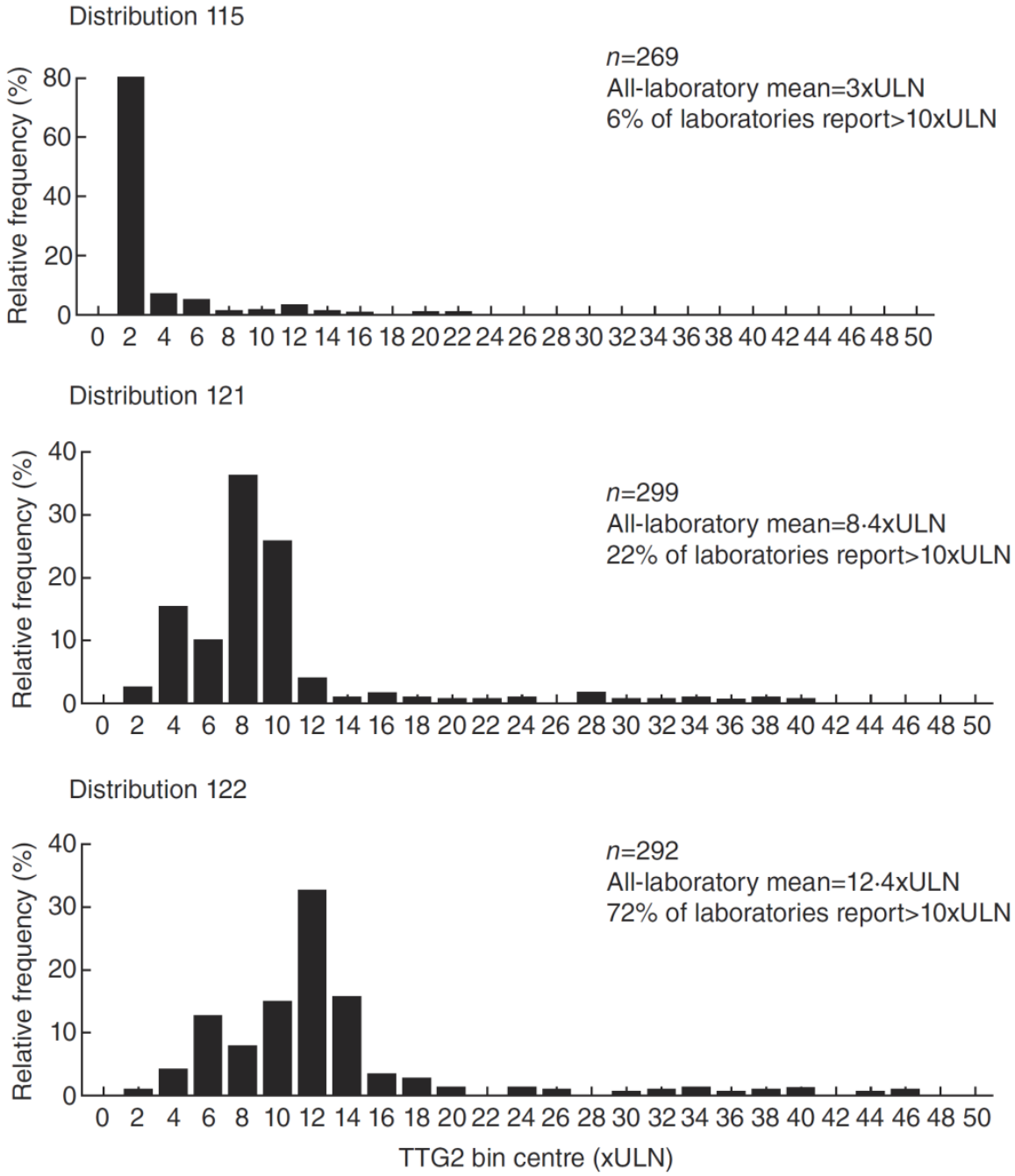
Positive Anti-TG2 <10x normal
EMA & HLA testing for DQ2/DQ8
Not available
OEGD & biopsies
EMA pos HLA pos
EMA pos HLA neg
EMA neg HLA neg
EMA neg HLA pos
Marsh 0-1
Marsh 2 or 3
CD+
Consider false neg. HLA test Consider biopsies
Consider false pos. Anti-TG2 test
Unclear case
Consider:
false pos. serology
false neg. biopsy or
potential CD
Extended evaluation of
HLA/serology/biopsies
CD+

	Method	Valid returns	All-laboratory mean (021×ULN)	Lowest (×ULN)	Highest (×ULN)	Proportion> 10 × ULN	Proportion< 10 × ULN
Distribution 115	All methods	269	3·0	1·5	21·8	6%	94%
	Inova	27	8·4	1·5	21·8	55·6%	44·4%
	Phadia 250	154	2·3	1·6	3·4	0·0%	100·0%
	Orgentec	41	1·5	1·1	1·8	0·0%	100·0%
	Euroimmun	17	5·1	3·7	7·1	0·0%	100·0%
	Phadia Varelisa	18	1·5	1·0	2·8	0·0%	100·0%
	Aesku	12	3·9	1·5	7·5	0·0%	100·0%
Distribution 121	All methods	299	8·4	1·6	39·7	22%	78%
	Inova	31	19·3	6·1	39·7	58·1%	41·9%
	Phadia 250	168	8·7	2·8	12·1	14·3%	85·7%
	Orgentec	43	4·7	3·0	7·4	0·0%	100·0%
	Euroimmun	20	10·0	7·3	28·0	95·0%	5·0%
	Phadia Varelisa	23	4·9	2·6	11·4	4·3%	95·7%
	Aesku	14	8·1	1·6	18·7	28·6%	71·4%
Distribution 122	All methods	292	12·4	1·4	45·3	72%	28%
	Inova	29	25·5	8·8	45·3	96·6%	3·4%
	Phadia 250	165	12·1	1·4	17·4	89·7%	10·3%
	Orgentec	42	6·0	3·3	9·4	0·0%	100·0%
	Euroimmun	19	14·0	10·0	44·5	100·0%	0·0%
	Phadia Varelisa	24	8·7	3·4	18·2	29·2%	70·8%
	Aesku	13	13·5	1·9	24·4	69·2%	30·8%

Beltran et al. Clin Exp Imm 2013;176:190

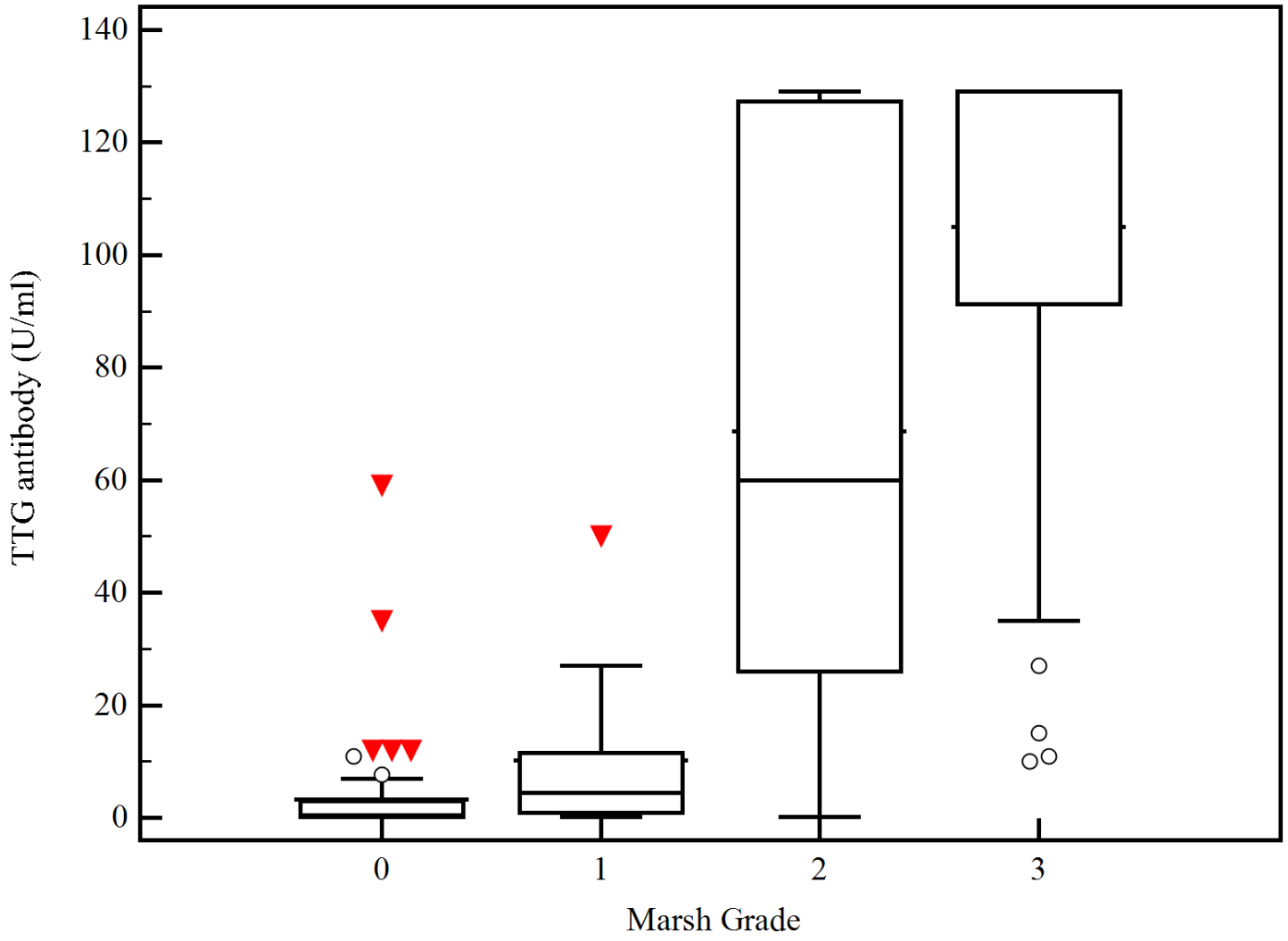
	Method	Valid returns	All-laboratory mean (021×ULN)	Lowest (×ULN)	Highest (×ULN)	Proportion> 10 × ULN	Proportion< 10 × ULN
Distribution 115	All methods	269	3·0	1·5	21·8	6%	94%
	Inova	27	8·4	1·5	21·8	55·6%	44·4%
	Phadia 250	154	2·3	1·6	3·4	0·0%	100·0%
	Orgentec	41	1·5	1·1	1·8	0·0%	100·0%
	Euroimmun	17	5·1	3·7	7·1	0·0%	100·0%
	Phadia Varelisa	18	1·5	1·0	2·8	0·0%	100·0%
	Aesku	12	3·9	1·5	7·5	0·0%	100·0%
Distribution 121	All methods	299	8·4	1·6	39·7	22%	78%
	Inova	31	19·3	6·1	39·7	58·1%	41·9%
	Phadia 250	168	8·7	2·8	12·1	[illegible]	85·7%
	Orgentec	43	4·7	3·0	7·4	0·0%	100·0%
	Euroimmun	20	10·0	7·3	28·0	95·0%	5·0%
	Phadia Varelisa	23	4·9	2·6	11·4	[illegible]	95·7%
	Aesku	14	8·1	1·6	18·7	28·6%	71·4%
Distribution 122	All methods	292	12·4	1·4	45·3	72%	28%
	Inova	29	25·5	8·8	45·3	96·6%	3·4%
	Phadia 250	165	12·1	1·4	17·4	[illegible]	10·3%
	Orgentec	42	6·0	3·3	9·4	0·0%	100·0%
	Euroimmun	19	14·0	10·0	44·5	100·0%	0·0%
	Phadia Varelisa	24	8·7	3·4	18·2	[illegible]	70·8%
	Aesku	13	13·5	1·9	24·4	69·2%	30·8%

Beltran et al. Clin Exp Imm 2013;176:190

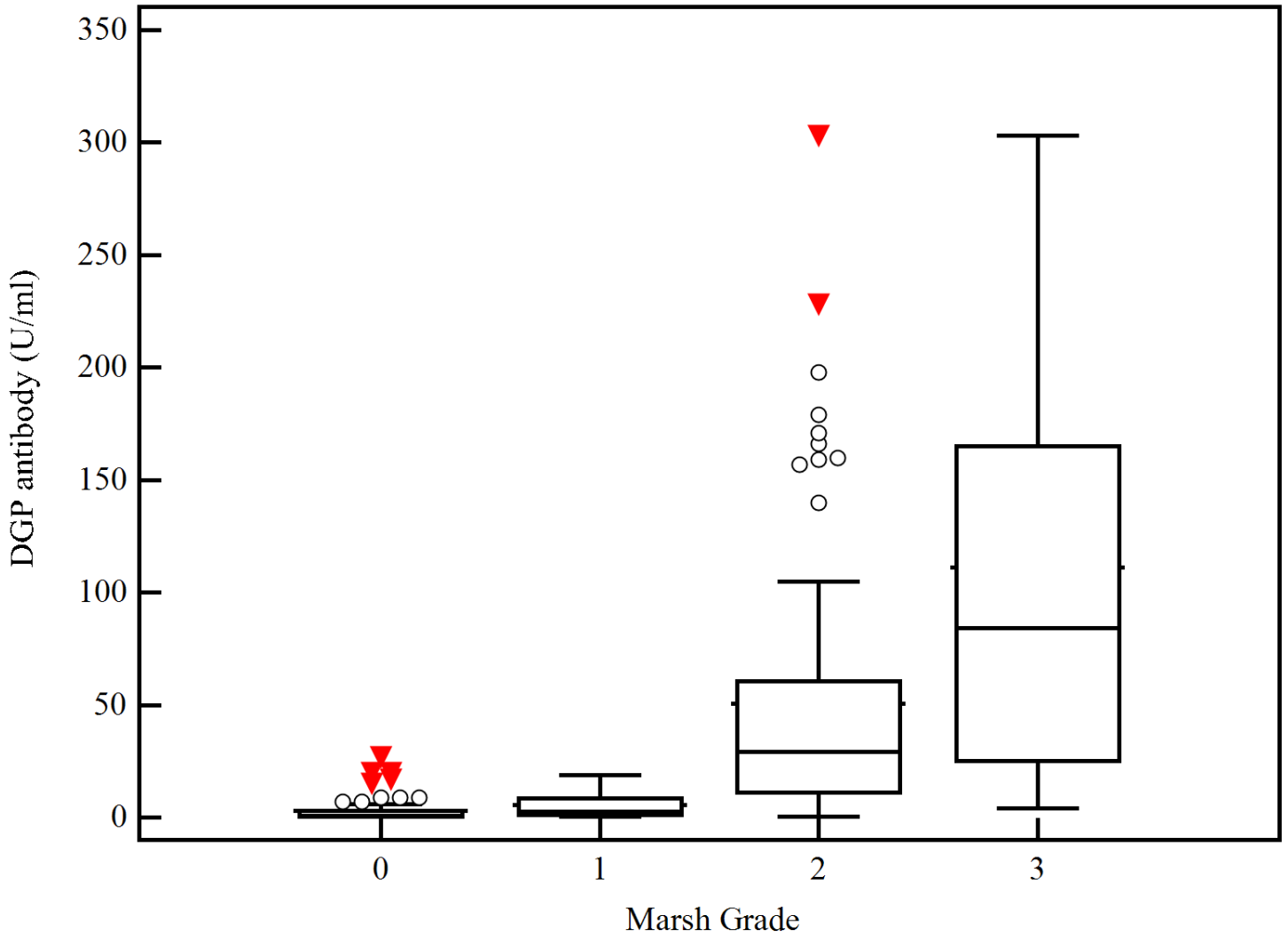


Beltran et al. Clin Exp Imm 2013;176:190

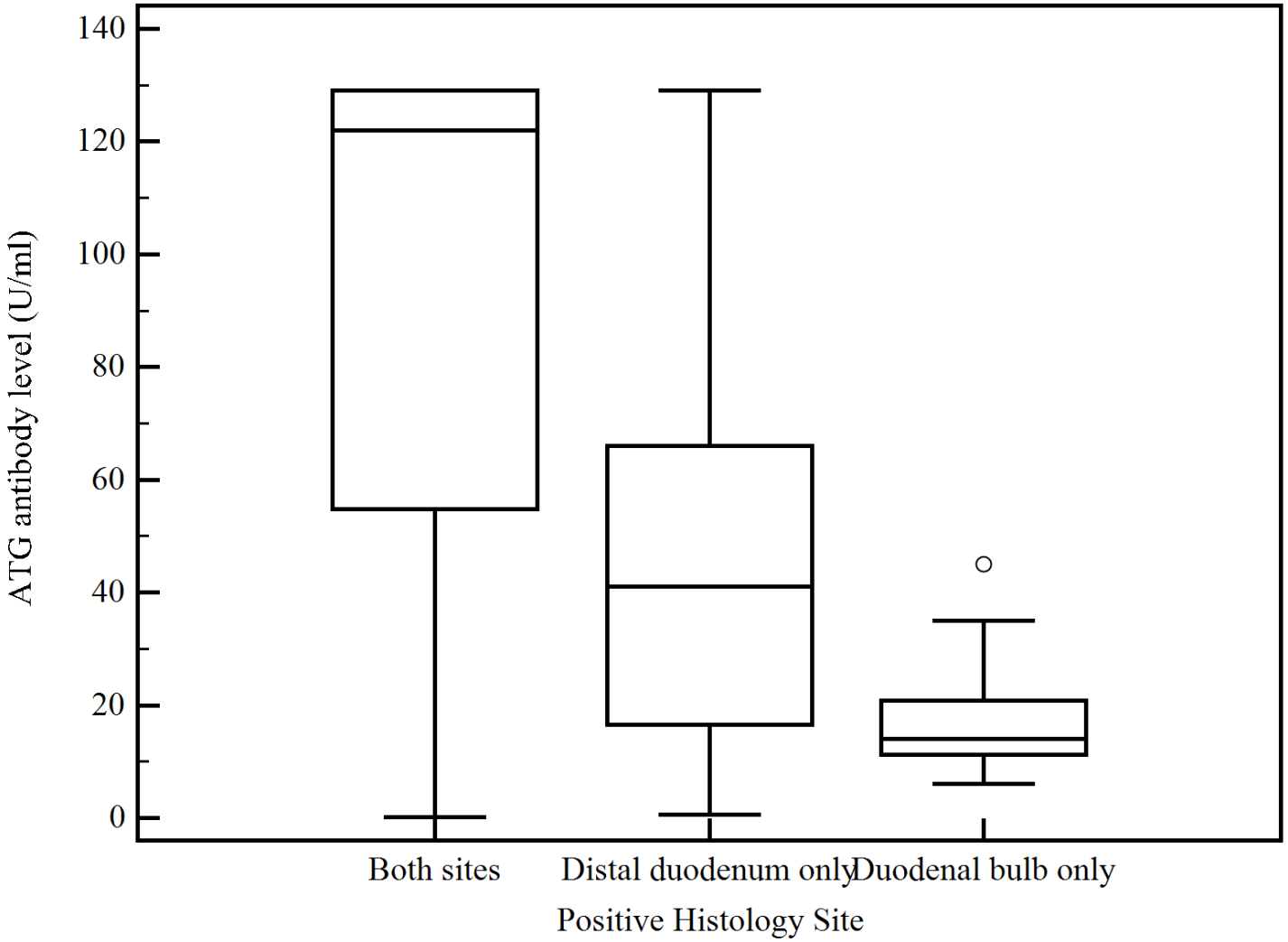
IgA TTG level correlates with histology



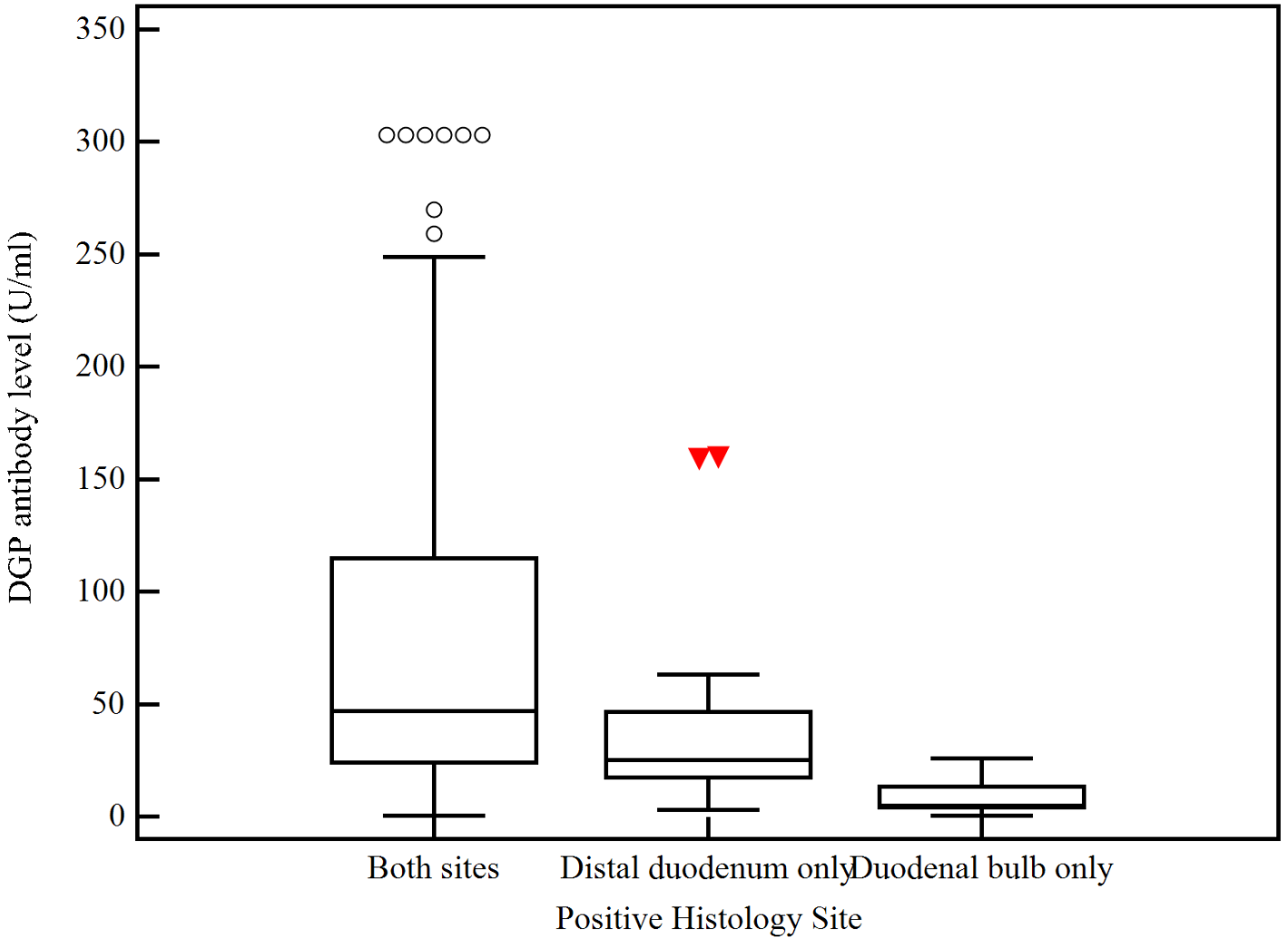
IgG DGP level correlates with histology



IgA TTG lower in single site positive



IgG DGP lower in single site positive



Common Variable Immunodeficiency (CVID)

- Relatively **common immunodeficiency** with **variable** levels of immunoglobulins and clinical course between patients
- Most frequent clinically symptomatic PID
- Prevalence between 1:10,000 and 1:50,000
- Some genes have been identified but most cases remain unidentified

CVID definition

- Low immunoglobulins of at least 2 isotypes (IgG and either IgA or IgM)
- Associated with poor specific antibody responses:
- Poor antibody response to vaccines and/or absent isohaemagluttinins
- Disease onset may occur at any age – most in adulthood

CVID definition

- Infections – most common (90%) with sinopulmonary, ear and gastrointestinal infections
- GI (up to 50%) – chronic diarrhoea, malabsorption
- Lymphophadenopathy or splenomegaly (50%)
- Autoimmunity (30%)
- Granulomas (10-30%) – lungs, liver, other
- Malignancy – increased incidence of lymphoma and gastric cancer

CVID enteropathy

- Seen in up to 10-30%
- Associated with atrophic gastritis 80%
- May have distinctive features:
  - Absence/paucity of intestinal plasma cells
  - Diffuse follicular lymphoid hyperplasia
  - GVH-like crypt apoptosis
  - Neutrophil infiltration
  - IEL infiltrate CD8+ T cell enriched

Malamut et al. Am J Gastro 2010; 105:2262-75

CVID enteropathy

- Serologic studies are not helpful
- May be presenting feature
- Small numbers improve on GFD
- Patients with severe disease may benefit from Budesonide