Frontiers in Celiac Disease:

Where Autoimmunity and Environment Meet

PRESENTED BY

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Definitions

Autoimmunity

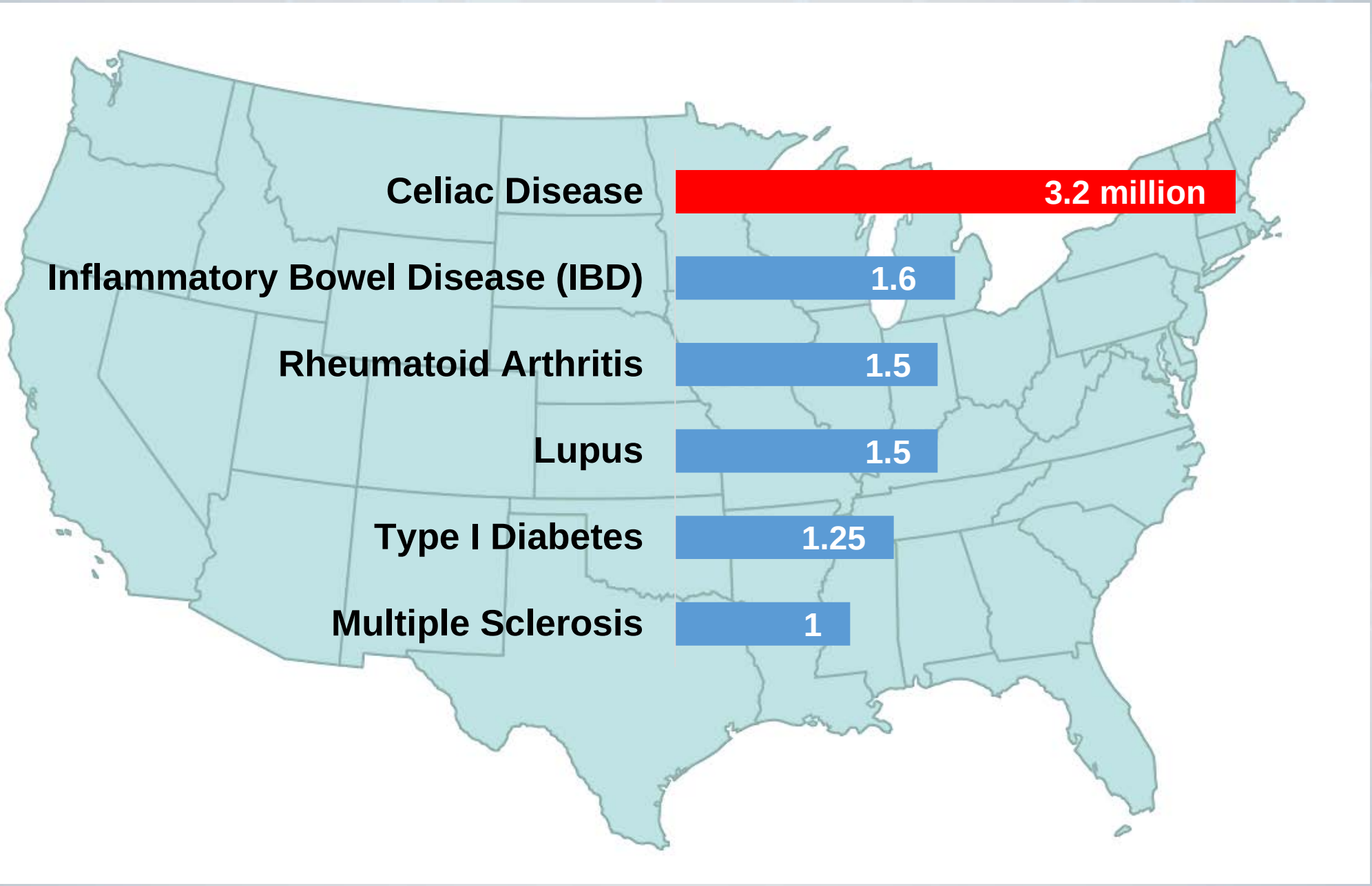
The system of immune responses of an organism against its own healthy cells and tissues.

Environment

The complex of physical, chemical, and biotic factors (such as climate, soil, and living things) that act upon an organism or an ecological community and ultimately determine its form and survival.

Environment of a Disease

- Biological environment
  - Genetics
  - Interface with other organisms
    - Symbiotic-microbiome
    - Pathologic- infections
- Dietary, Hygiene and Medication environment
  - Geographic and cultural differences
  - Antibiotic use practices
- Societal environment
  - How society views the importance of the disease
  - Level of education and awareness, rates and accuracy of diagnosis, research funding, medical and social support for the condition

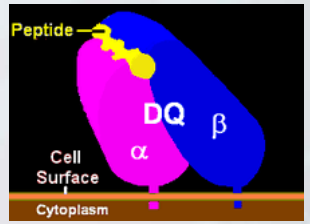

Celiac Disease
3.2 million
Inflammatory Bowel Disease (IBD)
1.6
Rheumatoid Arthritis
1.5
Lupus
1.5
Type I Diabetes
1.25
Multiple Sclerosis
1

Outline

- Overview celiac disease (CeD)
  - Epidemiology, clinical manifestations, burden of disease
  - Pathophysiology
- Duodenal pathology on biopsy at diagnosis and in follow up
- Clinical trials in celiac disease-a lot happening

What is Celiac Disease

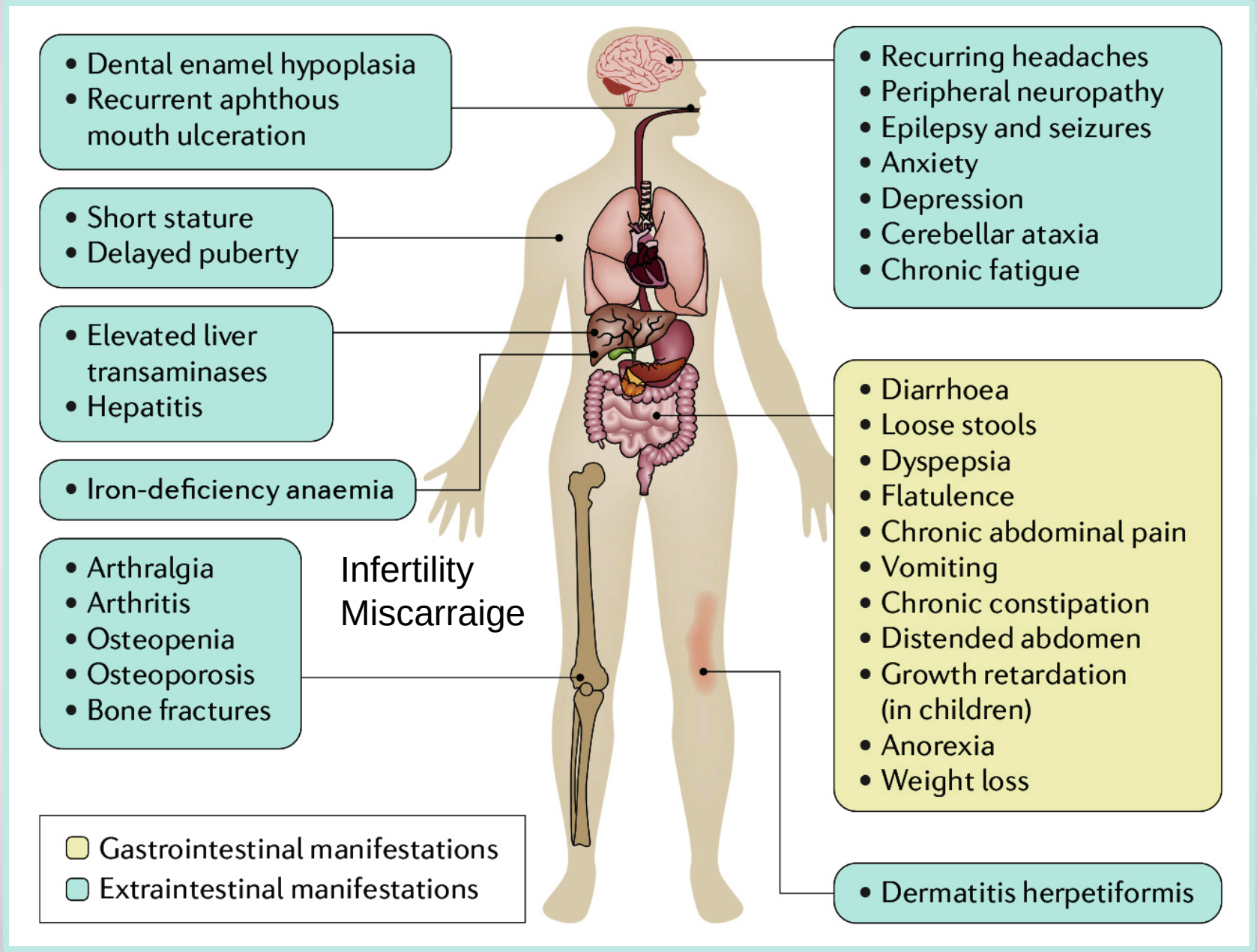
- Chronic, immune-mediated enteropathy driven by dietary gluten (wheat, barley and rye)
- More common in females, presents at any age and in almost all ethnicities
- Requires genetic susceptibility
  - Almost exclusively in MHC Class II HLA-DQ2 & DQ8 haplotypes
  - Other genetic factors
    - Only a fraction of DQ2/DQ8 develop disease
    - More than 100 non-HLA genetic associations
- Environmental factors
  - Amount and timing of gluten introduction into diet, microbiota, infections



While genetics provide the hard wiring, it is the environment that flips the switch on.

Protean Clinical Manifestations

- **May have <u>only</u> non-GI symptoms**
- **May be asymptomatic**



Perceived treatment burden is very high in treated Celiac disease

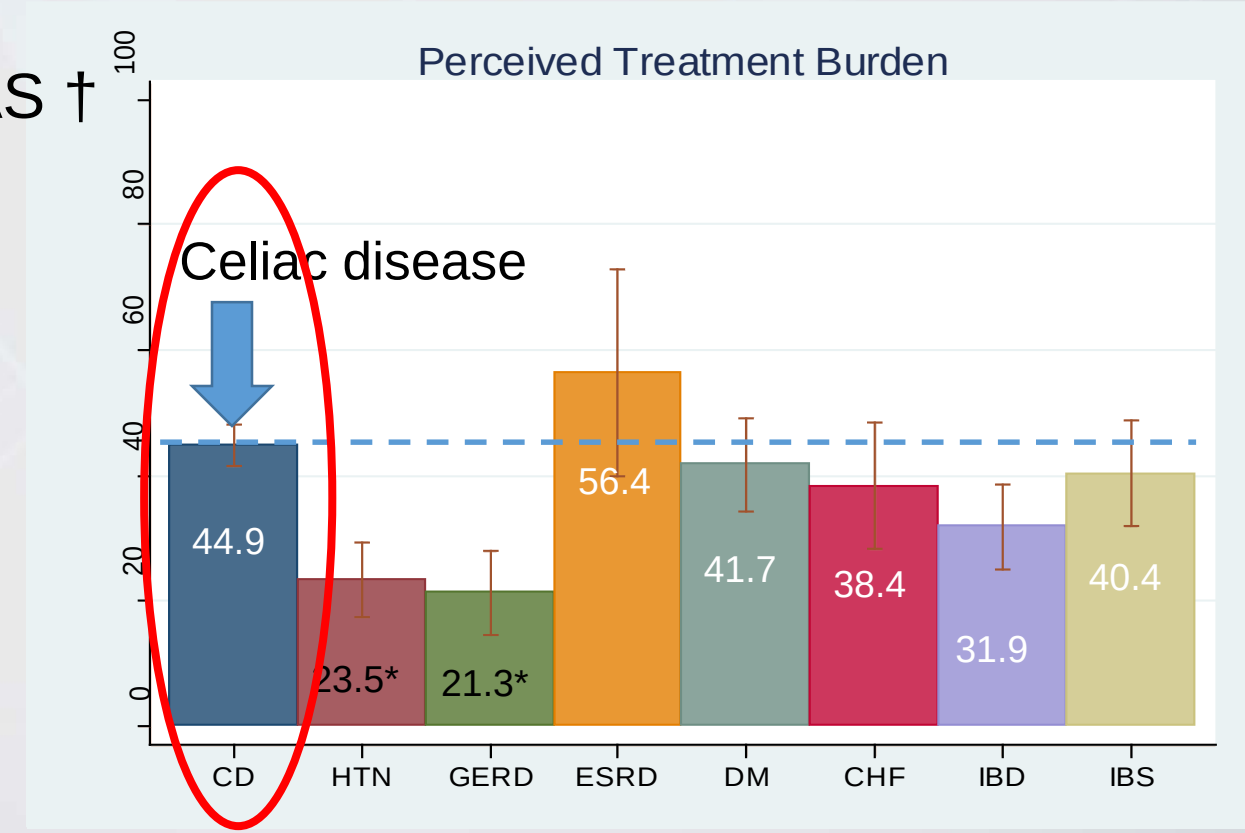
ESRD patients on hemodialysis = 56.4

Celiac disease = 44.9

-"Cloud over your head"
-Social anxiety/exclusion
-Lack of spontaneity
-Fear of eating out/travel
-Affects entire family
-"Not a real disease"
-Activities skipped
-Life decisions altered

A smaller, limited life

VAS †



† VAS: 0 = Very Easy
100 = Very Difficult

*Compared with CD, p<0.001

Shah S et al. Treatment burden in CeD. Am J Gastroenterol. 2014;109:1304-11.

Diagnosis

A significant barrier to diagnosis is the ongoing insufficient awareness among medical professionals

Adults report average delay of diagnosis of 6-10 years. Delays in children also occur.

- Serologic tests
  - Anti-tissue transglutaminase IgA antibodies
    - Quantitative IgA assay
  - Anti-deamidated Gliadin Peptide IgG antibodies
  - Anti-endomysial antibodies

- HLA typing- >95% harbor HLA DQ2 (90%) or DQ8

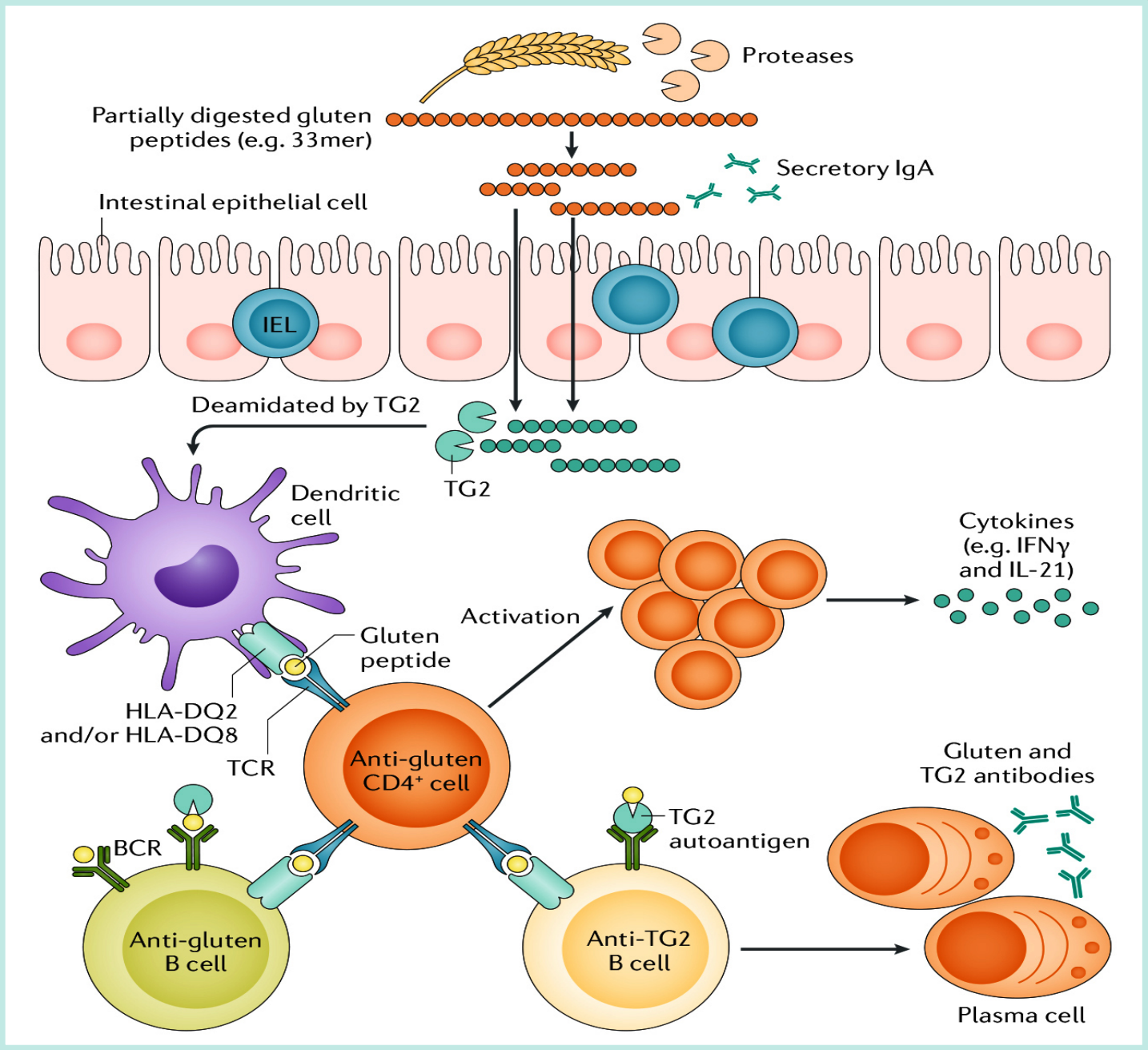
- Duodenal biopsies, 2 from bulb and 4 from second duodenum
  - Recently, may omit biopsy in children if certain criteria met

Pathogenesis

Adaptive immune responses in CeD

Characterized by:

- Binding of deamidated gluten to dendritic APCs
- Gluten-specific mucosal CD4+ T-cells
- Anti-gliadin and TG2 antibodies
- Secrete IFN and IL21
- Increase permeability of epithelial barrier



Innate Immune response

- IELs induce apoptosis
- Tregs suppressed

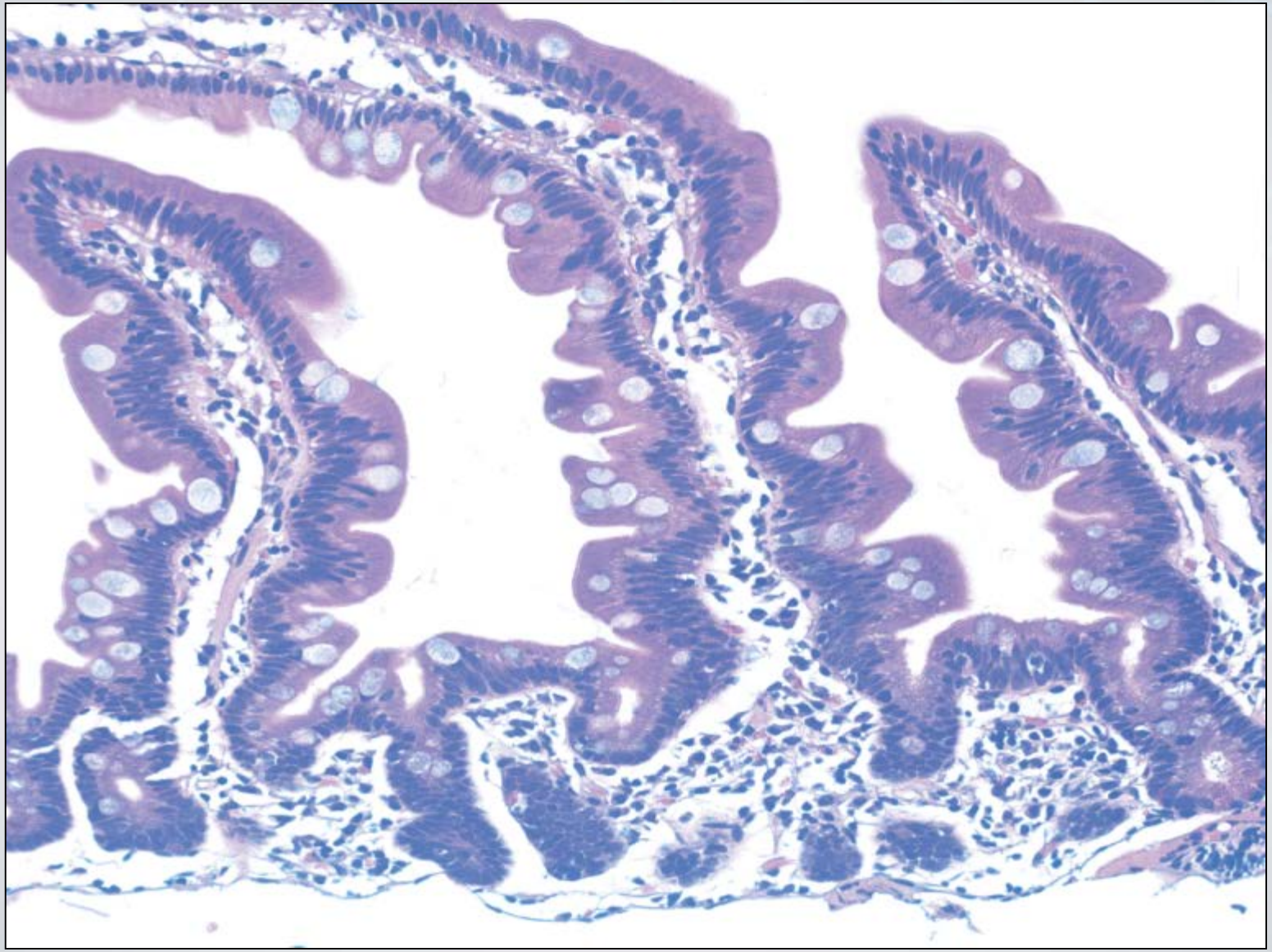
Lindfors et al, Nature Reviews/Disease Primers, (2019) 5:3.

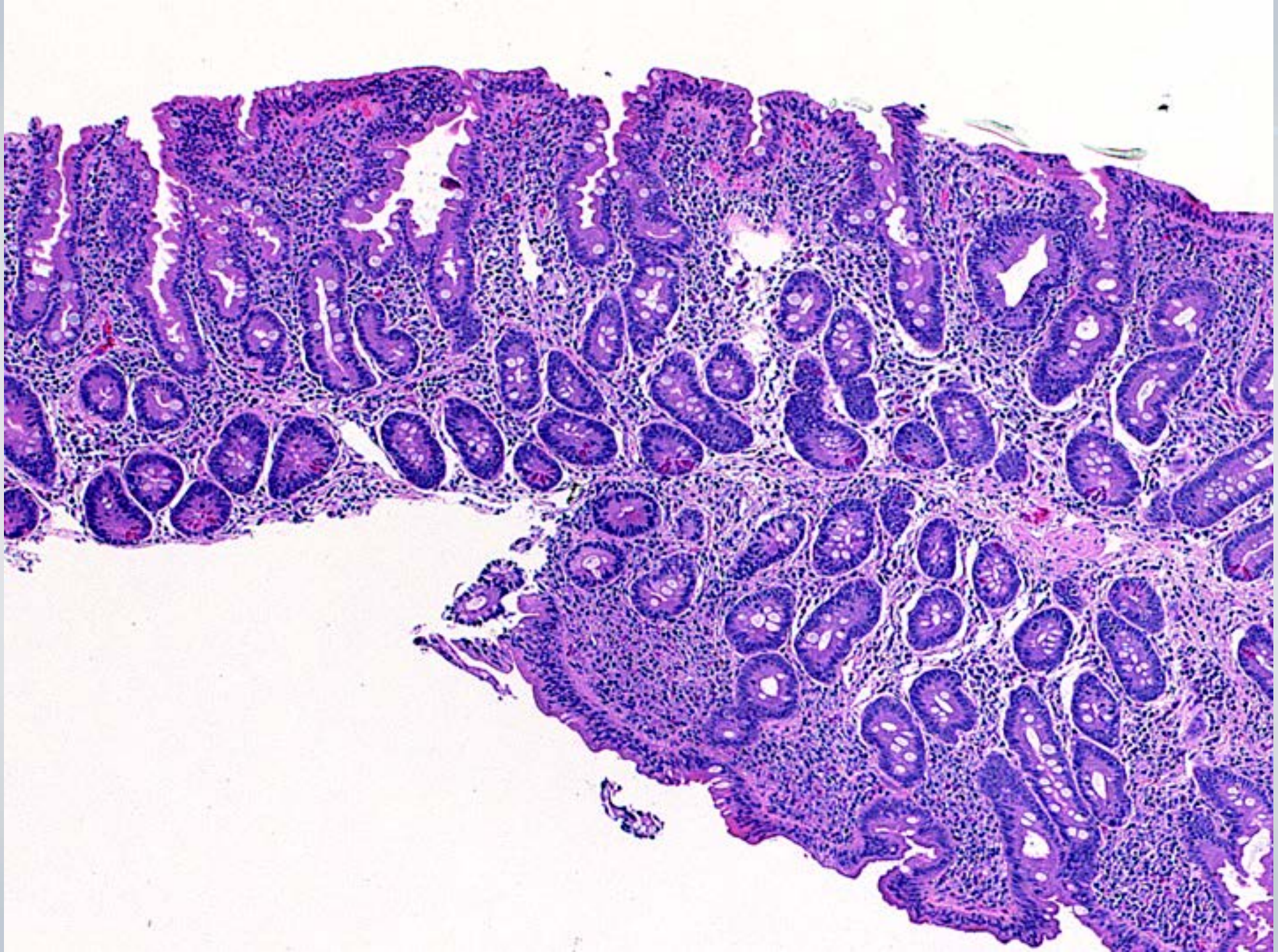
Role of Microorganisms in Celiac Disease: A Simplification

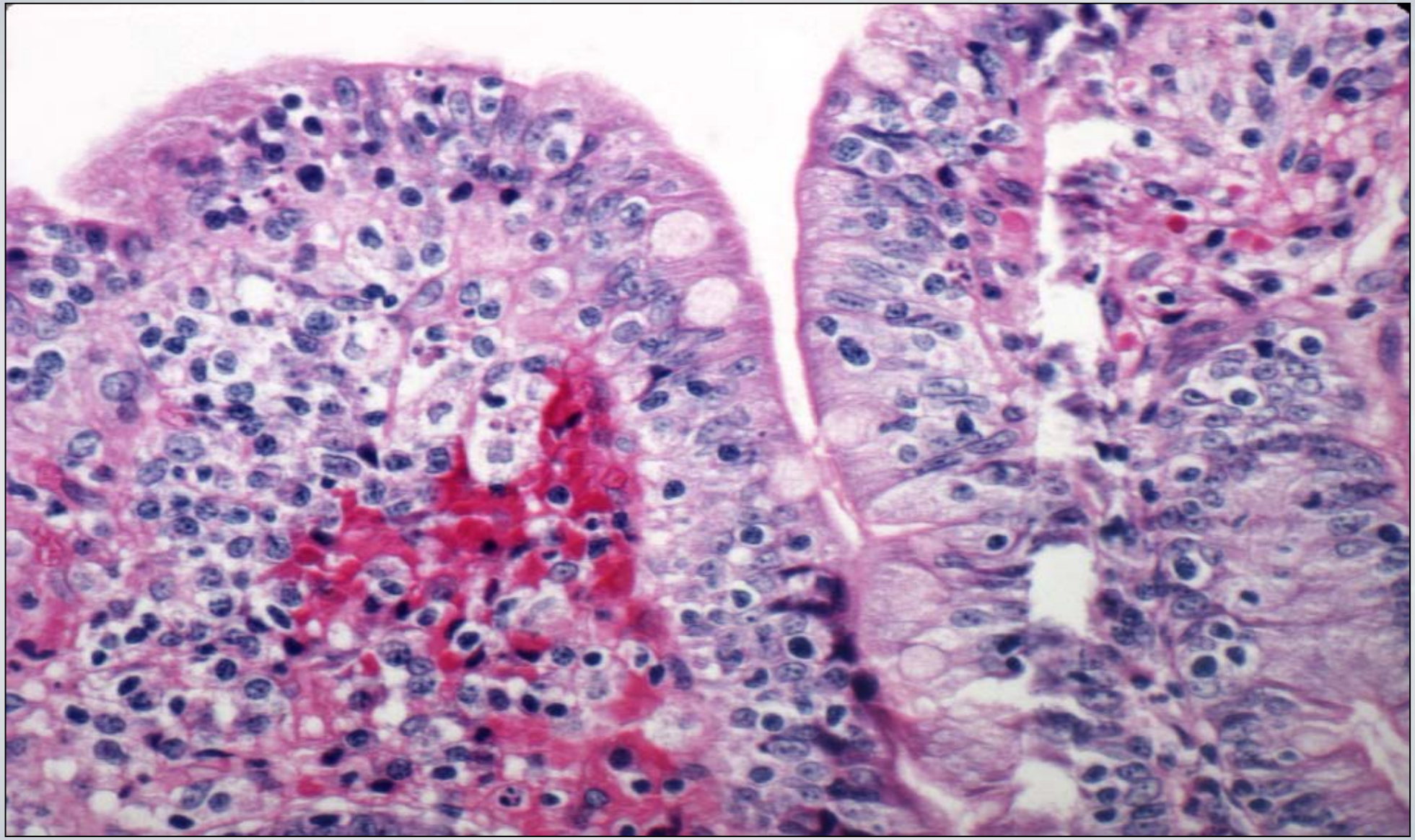
- In vivo and in vitro studies (mostly of bacteria) support association between gut microbiota and celiac disease
- Taxonomic microbiota composition differs between active CeD, GFD and normal controls, in oral, duodenal and fecal samples
  - Increase in virulent strains (D'Argenio et al. Am J gastroenterol. 111:879-890, 2016)
  - Can modify immunogenic food antigens, increasing or decreasing their immunogenicity
  - Use undigested particles as substrates, producing metabolites such as short chain fatty acids affecting homeostasis
  - Contribute to intestinal barrier dysfunction
- Common viral infections (rotovirus and rheovirus) trigger inflammatory response to gluten antigens by initiating T_H1 responses instead of Treg responses
  - Associated with development of symptomatic celiac disease in susceptible hosts.
    - Bouziat et al. Science 365:44-50, 2017

Duodenal Mucosal Pathology

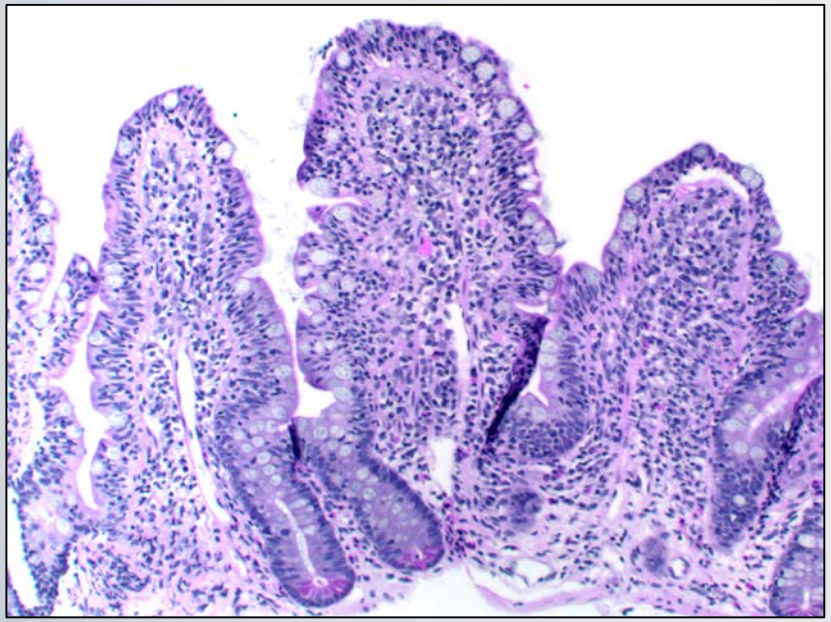
Non-Frontier Territory



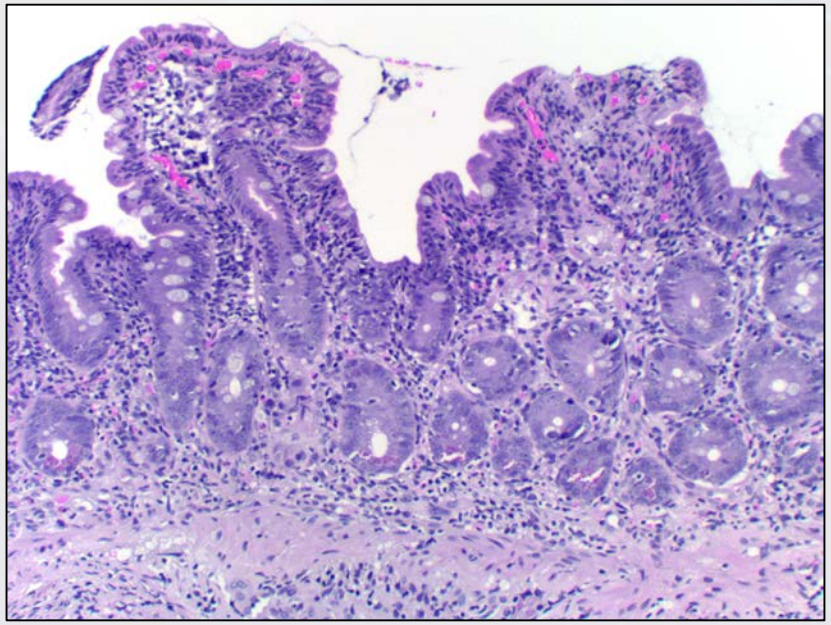




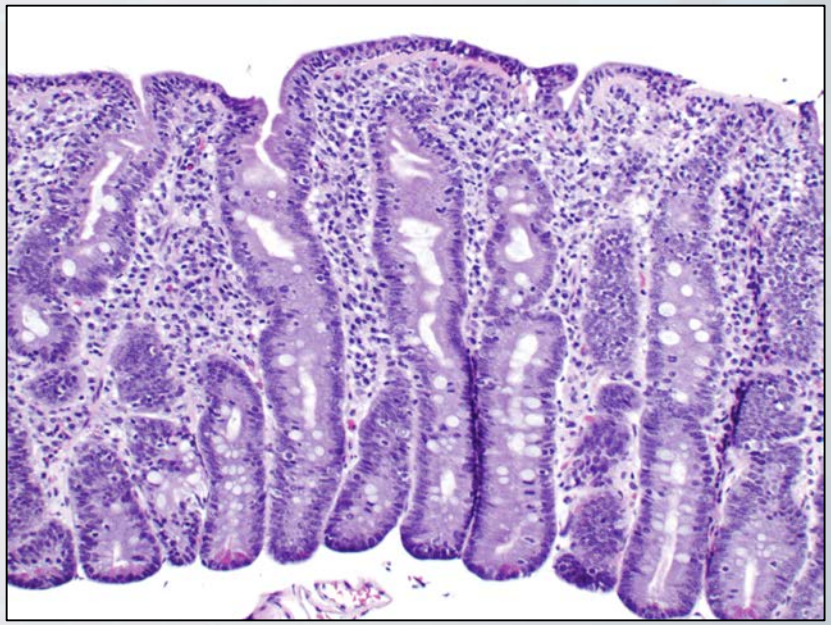
Villous blunting scores (Marsh System, 0-4)



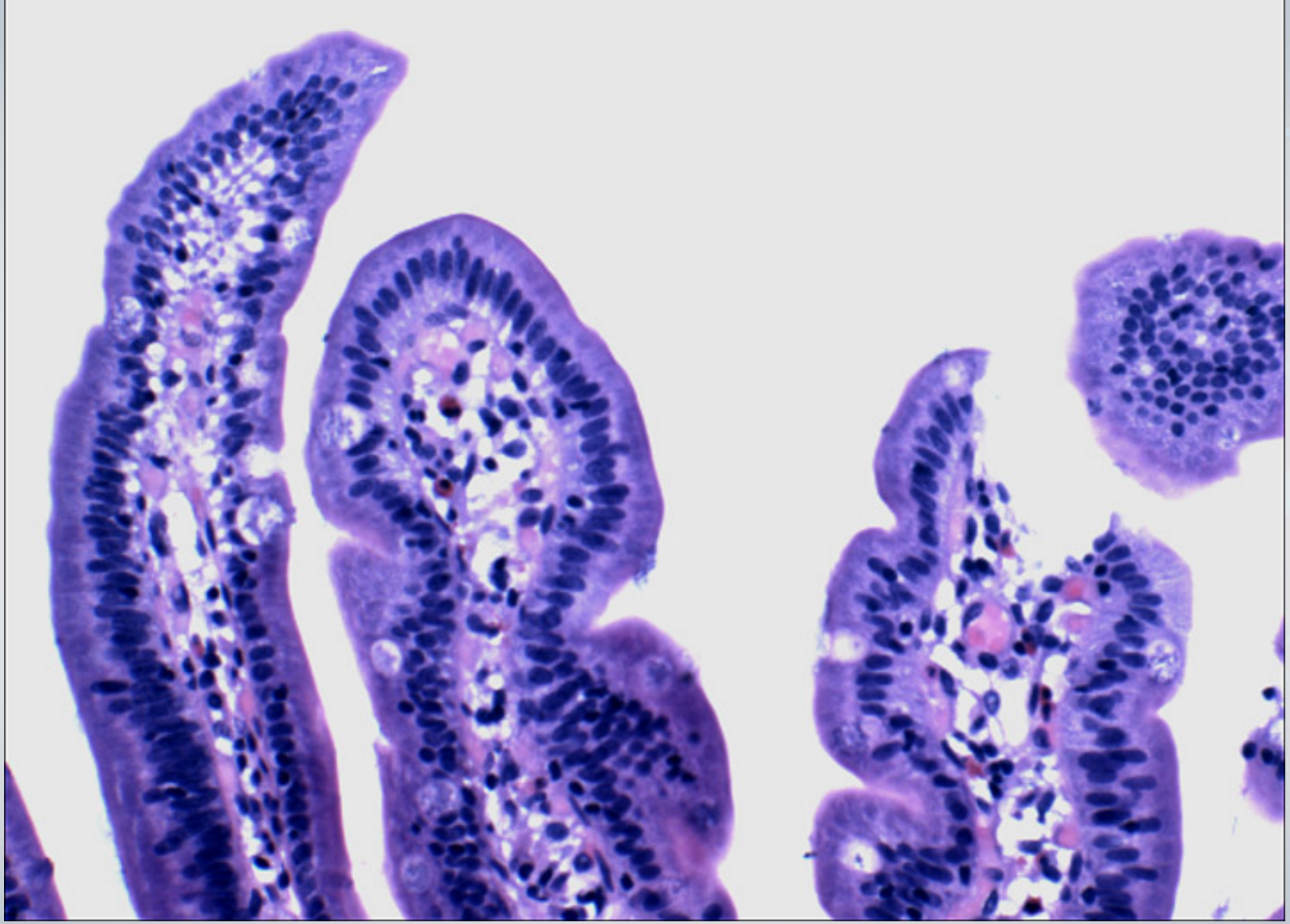
Mild blunting, Marsh 3A

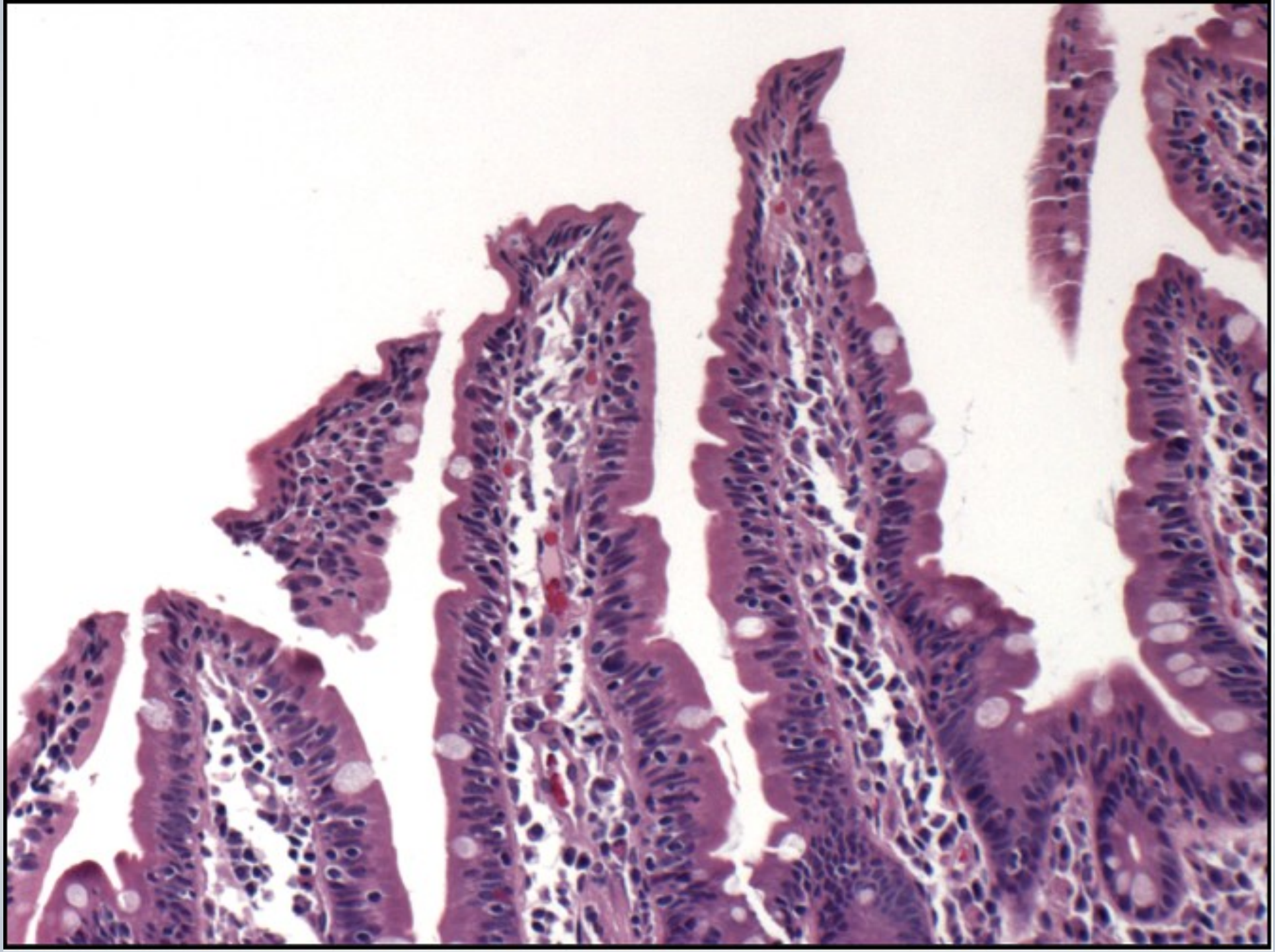


Moderate blunting, Marsh 3B



Severe blunting, Marsh 3C



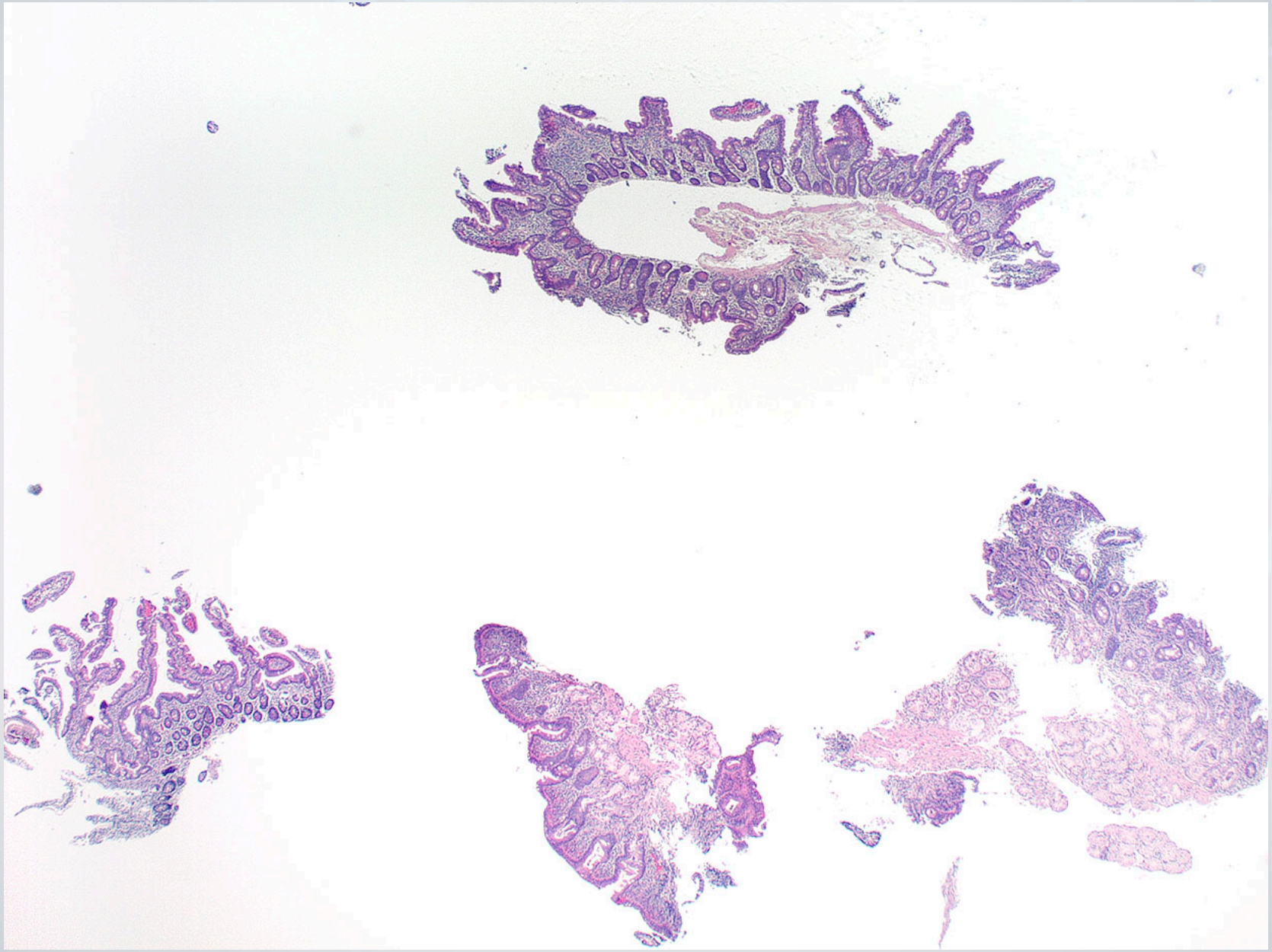


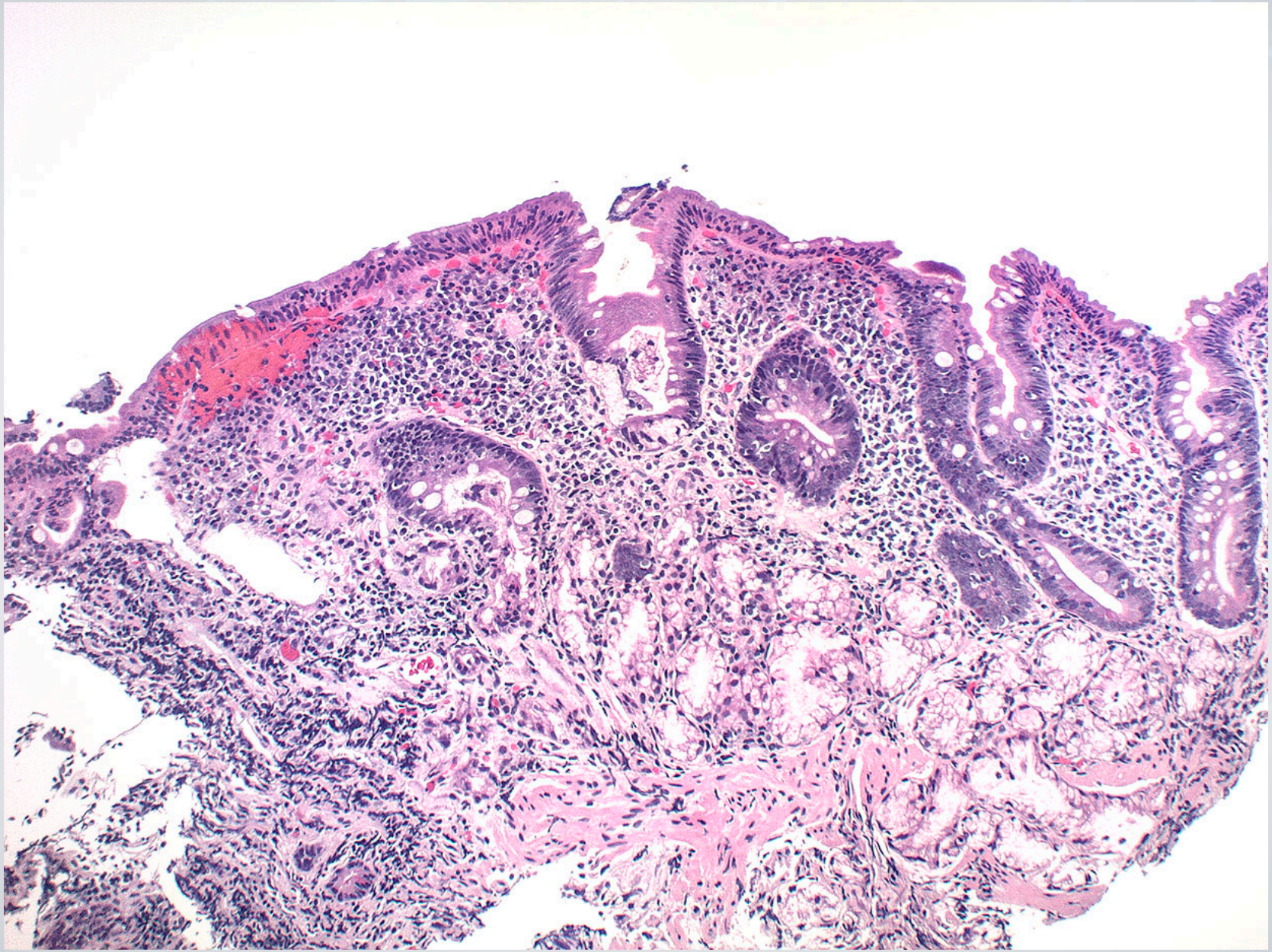
Assessing Intraepithelial Lymphocytes

Upper limit of normal is 20/100 enterocytes

- The villous tip method for use in normal villi
  - Jarvinen et al. Scand J Gastreoenterol 2004; , Biagi et al. J Clin Pathol 2004, Goldstein et al. Am J Clin Pathol 2001
- Villous tip to base ratio in 100 enterocytes at each site
  - Mino M et al.AJSP, 2003
- IELs in 50 enterocytes
  - Walker MM et al. Gastroenterol, 2010

- **Remember, increased IELs does not prove CD. Need serology!**





Evidenced based approach to duodenal sampling

- Duodenal bulb historically avoided due to common inflammatory changes attributed to ‘peptic duodenitis’
- Several studies confirm duodenal bulb reliably involved in celiac disease and may be only site of involvement
  - First described in children, then in adults
    - Gonzalez et al. Gastrointest Endosc 2010;72:1837-1842.
    - Evans et al. Am J Gastroenterol 2011;106:1837-1842.
    - Bonamico et al. J pediatr Gastroenterol nutr. 2008;47:618-622.
- AGA: Allen J, Katzka D, Robert M, Leontiadis G. Gastroenterol 2015;149:1088-1118.
- **GIPS/SSCD** joint recommendations
  - Robert ME, Crowe SE, Burgart L, Yantiss RK, Lebwohl B, Greenson JK, Guandalini S, and Murray JA. Am J Surgical Pathol. 42:e44-e58, 2018

Best Practices for Duodenal Biopsy in CeD:

Robert ME, Crowe SE, **Burgart L**, **Yantiss RK**, Lebwohl B, **Greenson JK**, Guandalini S, and Murray JA. Am J Surgical Pathol. 42:e44-e58, 2018.

A collaboration between GIPS and SSCD to address pathology component

- Practitioners should obtain at least 2 specimens from the proximal (bulb) and 4 from the distal duodenum

- The evaluation of villi must occur in well-oriented regions
  - Avoid lymphoglandular complexes and regions of where Brunner glands distort
  - Serial sections **should be employed routinely** in order to achieve well oriented villi for evaluation.
  - For diagnostic purposes, can decrease interobserver disagreement by considering **a three tier score: 'normal', 'partial' or 'complete'** blunting (Vh/Cd ratios in research)

- Intraepithelial lymphocyte quantity should be determined to be either within normal limits or increased
  - In equivocal cases, a quantitative assessment should be performed
  - **CD3 stain not needed**
  - **No need to include exact number in report outside of research setting**

Live Content Slide

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Poll: How should the duodenum be sampled to evaluate for the presence of celiac disease?

Answer

ARS 1: How should the duodenum be sampled to evaluate for the presence of celiac disease?

A. Four biopsies from the distal duodenum, since villous architecture cannot be reliably assessed in the duodenal bulb.

B. At least two biopsies from the duodenal bulb and four from the second duodenum.

C. Four biopsies from anywhere in the duodenum, since the disease can manifest in any segment.

Robert ME, Crowe SE, Burgart L, Yantiss RK, Lebwohl B, Greenson JK, Guandalini S, and Murray JA. Am J Surgical Pathol. 42:e44-e58, 2018.

Allen J, Katzka D, Robert M, Leontiadis G. Gastroenterol 2015;149:1088-1118

Duodenal Pathology: The Frontier

Is Follow Up Biopsy Needed in Celiac Disease

What is Proper Follow up for CeD Patients

Follow up often ‘Diagnose and Adios’, in adults

- Initial consult with dietician, then on your own
- No requirement of follow up biopsy to confirm return to ‘normal’ villous mucosa
- 10-30% have persistent symptoms despite good faith attempts at GFD
- Ongoing mucosal injury is associated with nutritional deficiencies, osteoporosis and lymphoma

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- Are symptom profile, GFD adherence, follow-up tTG titers surrogates for mucosal healing?
  - This premise has not been tested across celiac disease populations
  - Hint from clinical trials that mucosal injury persists in some patients
    - Follow biopsies are required in clinical trials (drug effect and endpoint)

- **Aims:**
  - Define factors associated with mucosal healing and persistent villous blunting in a multinational cohort
  - Stratify populations who may benefit from adjunctive therapies under development

N Patel[1], D Leffler[2], G Gan[1] ,Y Dan[1], A Atsawarungruangkit[2], A. Altoma[3] C Mulder[4] L Elli[5], A Del Gobbo[6] , J Goldsmith[7], Z Hintze[7], C Pacheco[8], M Vieth[9], B Melcher[9], M Salomao[10], R Pai[10], J Hart[11], A Olivas[11], B Naini[12], C Meyerson[12], W Choi[13], S Kakar[13], M Westerhoff[14], J Cheng[14], P Gopal[15], M Moreno[16], M Bronner[16], M Robert[1]

239 patients with 478 biopsies from two timepoints

184 patients with biopsy interval ≥ 1 year

Mean interval follow-up: 3.3yrs (range: 1-18yrs)

GFD Adherence:

Strict: 143 (78%)
Partial 34 (19%)
Absent: 5 (3%)

Classic Celiac Symptoms:

Diarrhea, stool incontinence, nausea, vomiting, fatigue, abdominal pain, bloating, flatus, constipation, dehydration, soft stool, urgency

Malabsorptive Symptoms:

Weight loss, loss of appetite, anorexia, cachexia, iron deficiency, anemia, vitamin B12 deficiency, steatorrhea, short stature, slow growth, hypoalbuminemia

CHANGES IN SYMPTOMS, TTG SEROLOGY, AND GFD DO NOT SIGNIFICANTLY CORRELATE WITH BIOPSY FINDINGS AT TIME POINT 2

		Distal			
		Marsh Score* N, (%)		IEL/100 enterocytes* N, (%)	
		Improved (N=130)	Did not Improve (N=40) N, (%)	Normalized (N=68) N, (%)	Elevated (N=91) N, (%)
Classic Celiac	Yes	56 (43%)	21 (53%)	24 (35%)	48 (53%)
	No	46 (35%)	15 (38%)	26 (38%)	29 (32%)
Malabsorptive	Yes	19 (15%)	9 (23%)	4 (6%)	22 (24%)
	No	32 (25%)	5 (13%)	21 (31%)	16 (18%)
Diarrhea	Yes	18 (14%)	11 (28%)	8 (12%)	20 (22%)
	No	33 (25%)	6 (15%)	19 (28%)	19 (21%)
Abdominal pain	Yes	33 (25%)	11 (28%)	16 (24%)	25 (27%)
	No	23 (18%)	7 (18%)	13 (19%)	12 (13%)
TTG Titer	Decrease	56 (43%)	13 (33%)	30 (44%)	36 (%)
	Did Not Decrease	36 (28%)	19 (48%)	13 (19%)	35 (%)
GFD	Strict	108 (83%)	24 (60%)	62 (91%)	59 (65%)
	Partial or No	21 (16%)	16(40%)	5 (7%)	32 (35%)
* P value = not significant for any parameter					

Initial Take Home Points

In a multinational pathology-based cohort of adults and children with celiac disease:

- Symptom improvement and decreasing tTG titers are not reliable indicators of normalization of duodenal histology in celiac disease.

- Duodenal mucosal injury persists in a significant subset of celiac disease patients adhering to a strict gluten free diet.

- These findings support the need for:
  - Greater monitoring of mucosal healing in celiac disease
  - Development of disease activity biomarkers that are less dependent on patient reported symptoms and diet adherence.

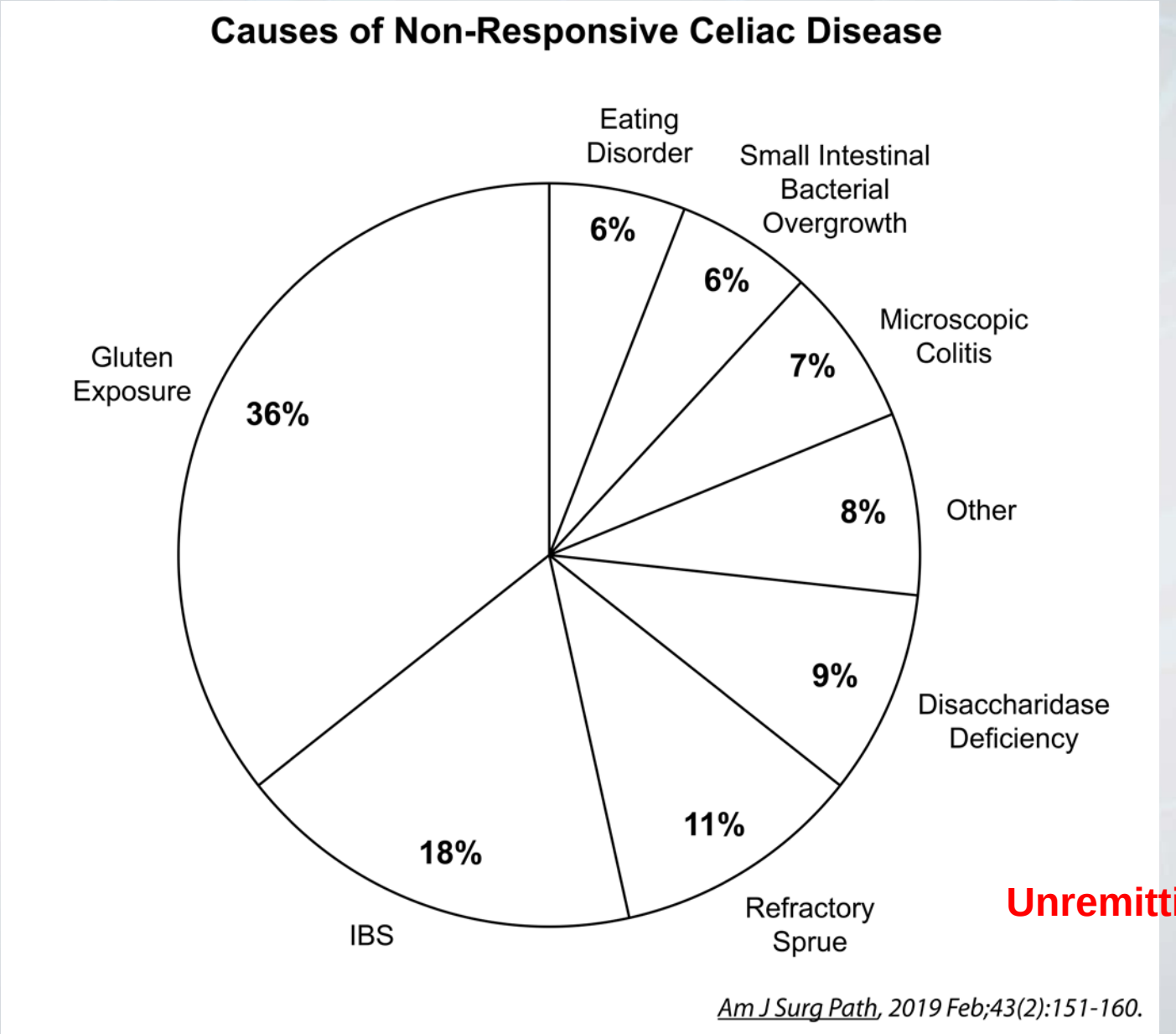
Refractory Celiac Disease

Definition Refractory Celiac Disease

Persistent or recurrent malabsorptive symptoms and signs with villous atrophy despite a strict gluten free diet for more than 12 months in the absence of other causes of non-responsive treated celiac disease and overt malignancy.

Ludvigsson, Leffler et al, GUT 2012

“Apparent Refractoriness”



Unremitting IL-15 production

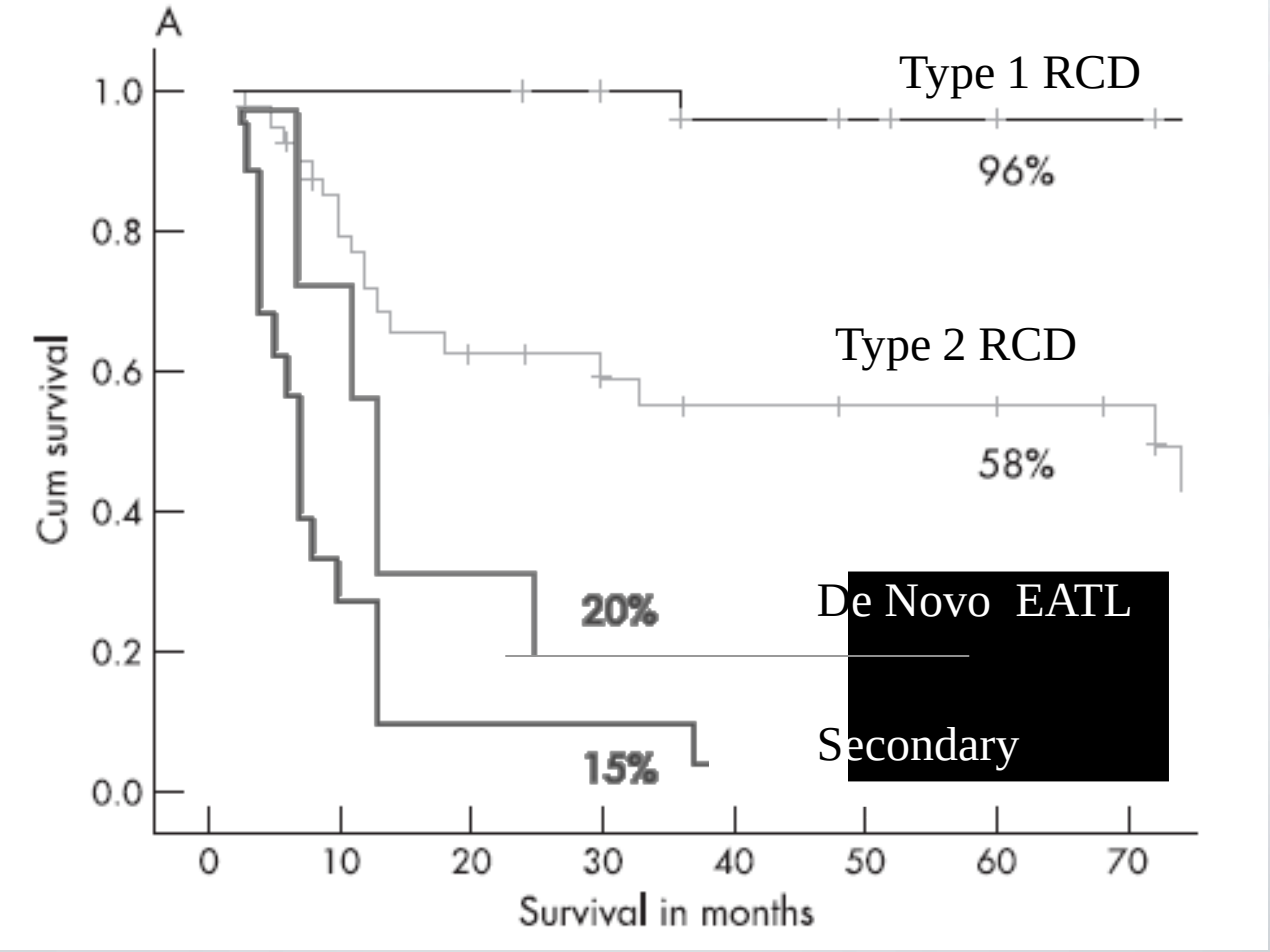
Two Categories of RCD patients
(defined at RCD referral centers)

Clinical/Pathologic Criteria	Disease Category	
	RCD type 1	RCD type 2
Abnormal intraepithelial lymphocyte immunophenotype: either >40-50 % by immunohistochemistry or >20-25% by flow cytometry	No	Yes
T-cell receptor chains (γ or δ) clonal rearrangement by molecular methods	No	Yes
Clinical or histological response to treatment	Yes	Variable
Lymphomagenesis potential (especially EATL development)	Rare	Frequent

Rubio-Tapia/Murray, Gut 2010

Prognosis by Refractory Celiac Disease Classification

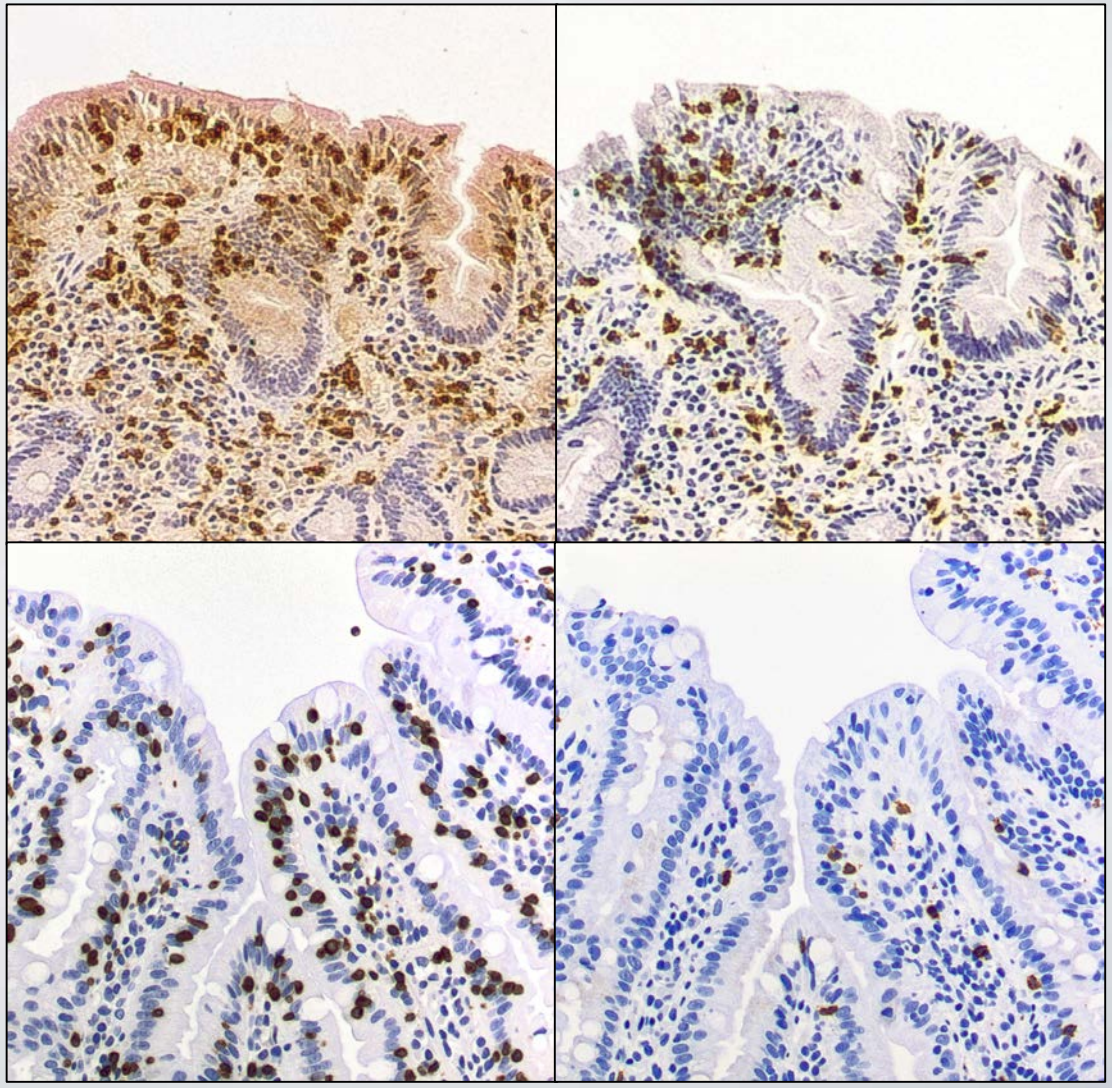
Al-toma et al, Gut 2007



Abnormal IELs (CD3ε+ CD8-) in refractory celiac disease

Type 1
Refractory CeD

Type 2
Refractory CeD



CD3 CD8

Cellier et al, Lancet 2000

Problems with Published RCD Diagnostic Criteria: The Yale Experience

- Following published guidelines, increase in requests for clonal analysis in follow up biopsies of symptomatic patients
  - Greater than 60% had monoclonal TCR gene rearrangements by PCR
  - No correlation with degree of villous blunting
  - Most patient's persistent symptoms responded to diet improvement
- Alarming result for physicians and patients
- Suspicion that test overly sensitive and lacking in clinical relevance
  - Had not been discussed in RCD literature

Clinical significance of monoclonal T-cell populations by PCR in celiac and non-celiac populations

Patient group	Number patients with T-cell clone	Villous architecture (some patients had multiple biopsies)	Clinical F/U of patients with clones
RCD2	**3/3**	4 severe	1 died of disease 2 clinical trials
RCD1	**4/4**	6 normal 1 mild 2 moderate 1 severe	4 alive and well on GFD
CeD-Follow up Bx	**7/10**	3 normal 2 mild 1 moderate 1 severe	1 asymptomatic w/ benign stricture 3 doing well, partial diet 2 not following diet 1 died of other disease
New Dx CeD	**2/10**	1 normal 1 severe	1 with diarrhea (IBS-like), on GFD 1 well on GFD
H.Pylori duodenitis	**3/5**	3 normal	N/A

Clonal T-cell populations clinically insignificant unless bona fide RCD criteria are met. Not previously reported.

Paradigm shift in Practice:
Resist submitting small biopsies for PCR analysis until the much more common causes of ‘apparent refractoriness’ are excluded.

Most prior literature subject to referral center bias.

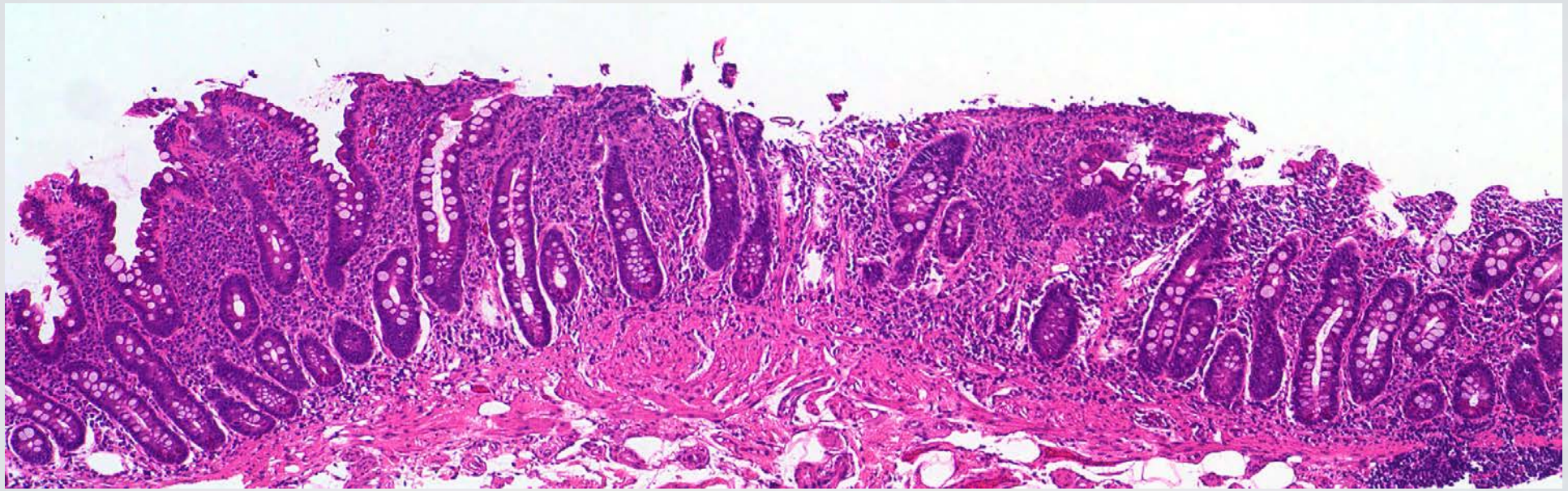
Let clinical features guide approach.

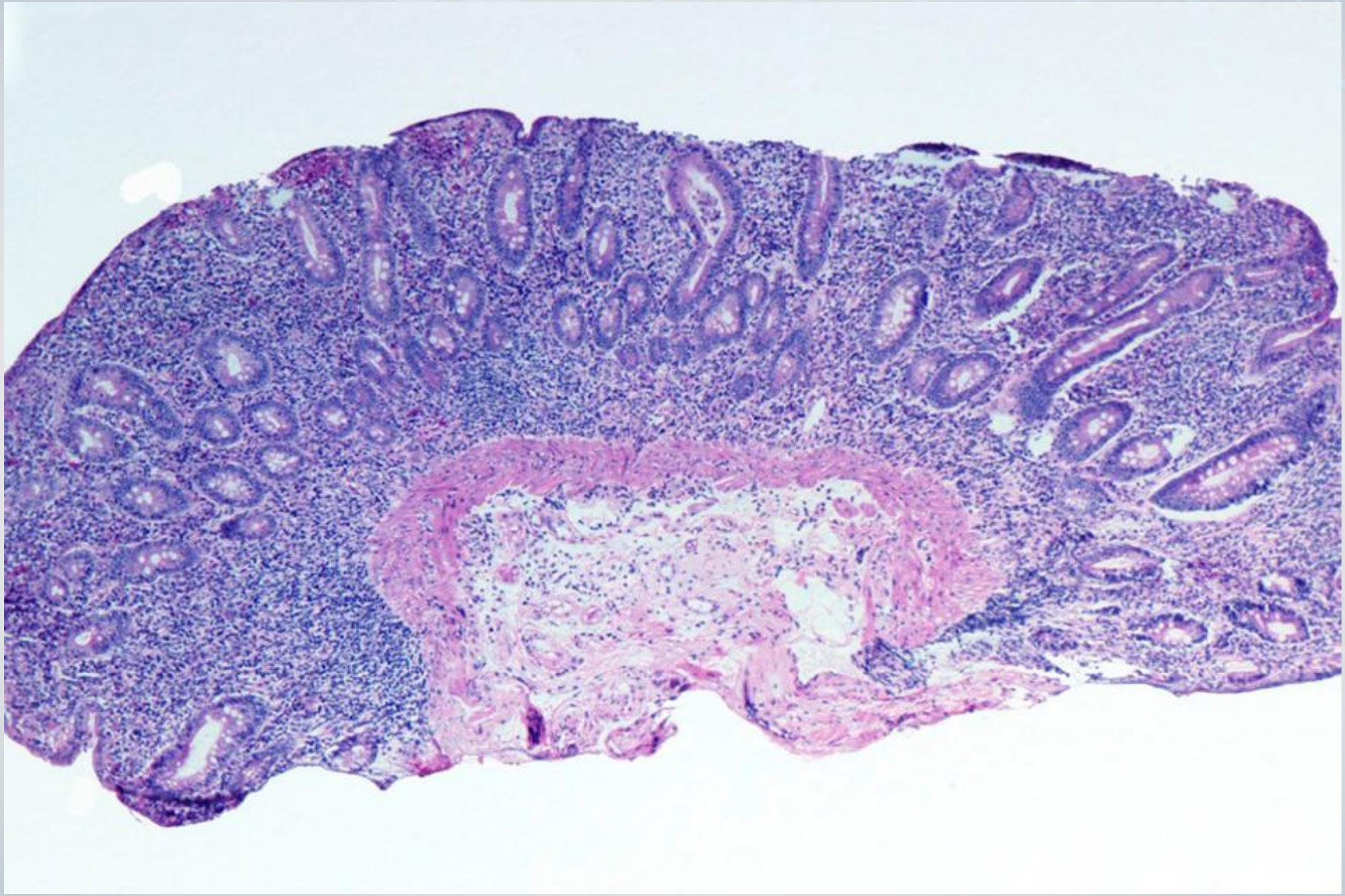
Celli, Hui, Triscott, Bogardus, Gibson, Hwang, Robert. Am J Surg Pathol 2019;43:151–160

Small Intestine Histology RCD

- Subcryptal lymphocytic infiltrates, 10/10
  - IBD like
- Thin mucosa/macrocytosis, 3/10
- Small bowel lymphoma, B-cell, 1/10
- Acute inflammation/ulceration, 5/10
- Collagenous sprue, 5/10 patients
  - Collagenous colitis in 2/5

Robert ME, Ament M, Weinstein W. Am J Surgical Pathol;24:676-687, 2000





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Poll: When is it appropriate to initiate a tissue work up for suspected RCD?

Answer

ARS 2: When is it appropriate to initiate a tissue work up for suspected RCD?

A. When follow up duodenal biopsies show persistent villous blunting in a celiac disease patient on a strict gluten free diet.

B. When clinician asks for T-cell clonal status and IHC phenotyping of intraepithelial lymphocytes in a duodenal biopsy.

C. When clinician has convincingly excluded likely causes of 'apparent refractoriness' in a celiac disease patient.

Celli, Hui, Triscott, Bogardus, Gibson, Hwang, Robert. Am J Surg Pathol 2019;43:151–160

Clinical Trials in Celiac Disease

Celiac disease:
Poised for drug development

Common: Revised prevalence estimates

- US ~0.02% [1/5000] revised to ~1% (~3 million in US]
- Europe ~0.1% [1/1000] revised to 1-2% (7-14 million)

GFD Inadequate:

- >10% Persistent / frequent non-responsive disease
- 1 - 2% Refractory to GFD
- ~ 30% of adults on GFD for celiac disease have ongoing partial villous atrophy on biopsy
- Strict GFD difficult to maintain

Need for **lifelong therapy**

Pathophysiology well elucidated - Multiple treatment targets

Progress in key areas

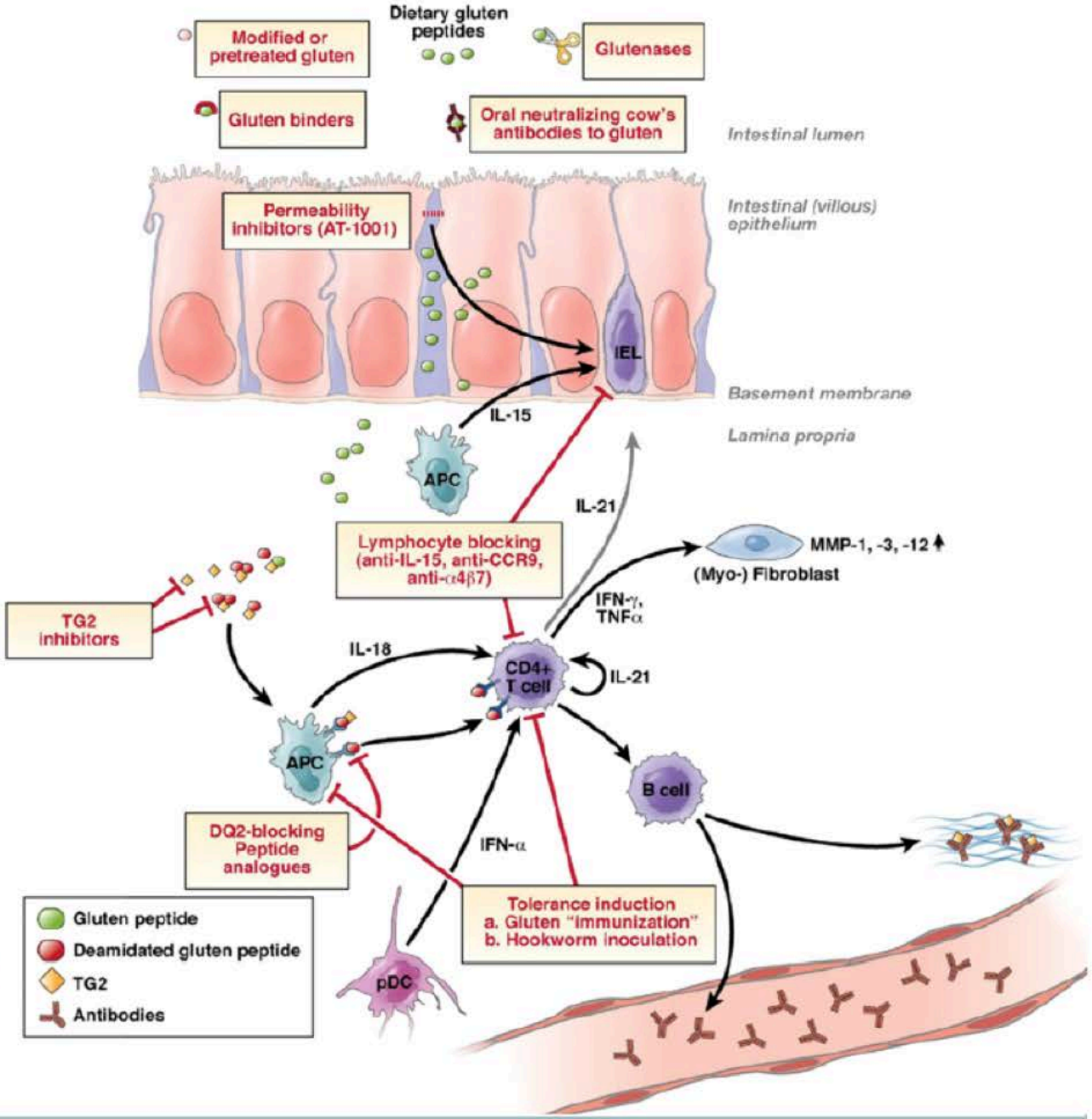
- Elucidation of acceptable study designs in path to approval
- Elucidation of acceptable outcome measures
- Success in recruiting volunteers to participate in clinical studies
- Robust co-operation by all stakeholders (patients, pharma, healthcare/research community and regulatory authorities (FDA, NIH).

Kelly CP, et al. Gastroenterol. 2015

Steps in Celiac Disease Pathogenesis

Gluten / gliadin

1. Ingested
2. Survives digestion
3. Crosses gut lining
4. “Made tastier” by TTG
5. Taken up by “antigen presenting cells” (APCs)
6. Genetically encoded DQ2 or DQ8 present
7. Presented on DQ2/8
8. T cells activated
   - Inflammation
   - Antibody production
   - Tissue damage



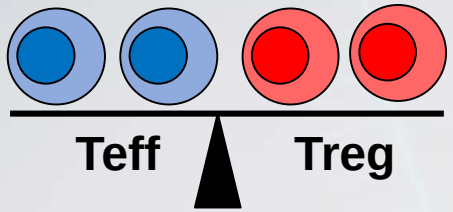
Schuppan et al. Gastroenterol 2009; 137:1912-33

Immune Tolerance

- Immunologic Tolerance – antigen-specific inhibition of selective immune responses is the ‘holy grail’ and the ‘future’ for the treatment of autoimmune diseases, allergy and tissue transplantation

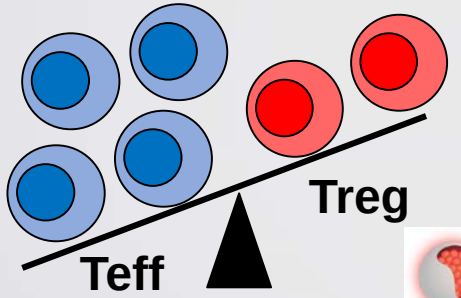
- Goal – ‘cure’ disease by specifically targeting ‘only’ the immunopathologic T cells in autoimmune disease rather than large scale immunosuppressive drugs which can lead to increased rates of infection and neoplasia

Immune Tolerance Therapy for Celiac Disease – Nanoparticle Technology –

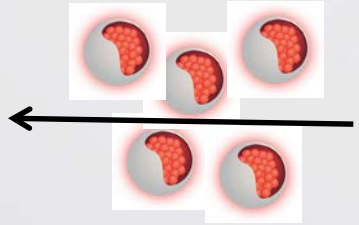


Healthy Individual – Balance between effector T cells (Teff) and regulatory T cells (Treg)

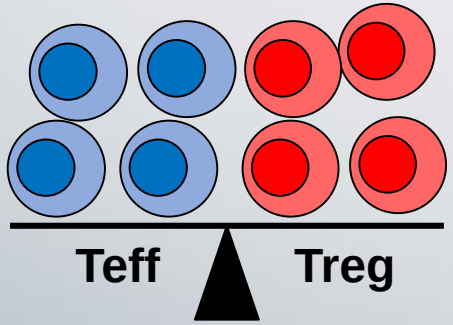
Gluten Sensitization



Celiac Disease – Autoimmune Teff cells outnumber the Treg cells leading to intestinal inflammation



Tolerogenic Immune Modifying Particle (TIMP) Infusion



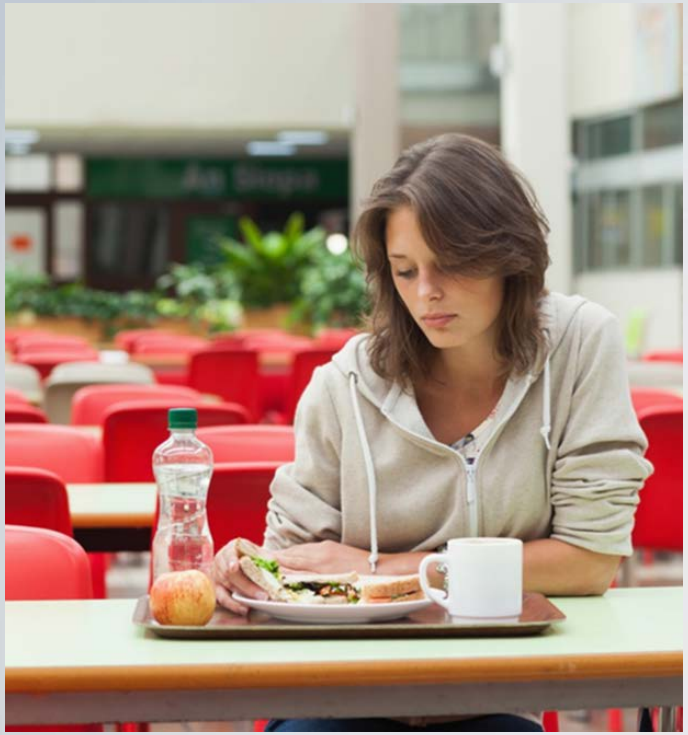
Tolerized Celiac Patient – PLG(Gliadin)-induced **expansion of gliadin-specific Tregs** restores balance between Teff cells and Tregs restoring homeostasis in the immune system and ameliorating disease symptoms

CNP-101 gliadin nanoparticles infusion (Phase 2a):

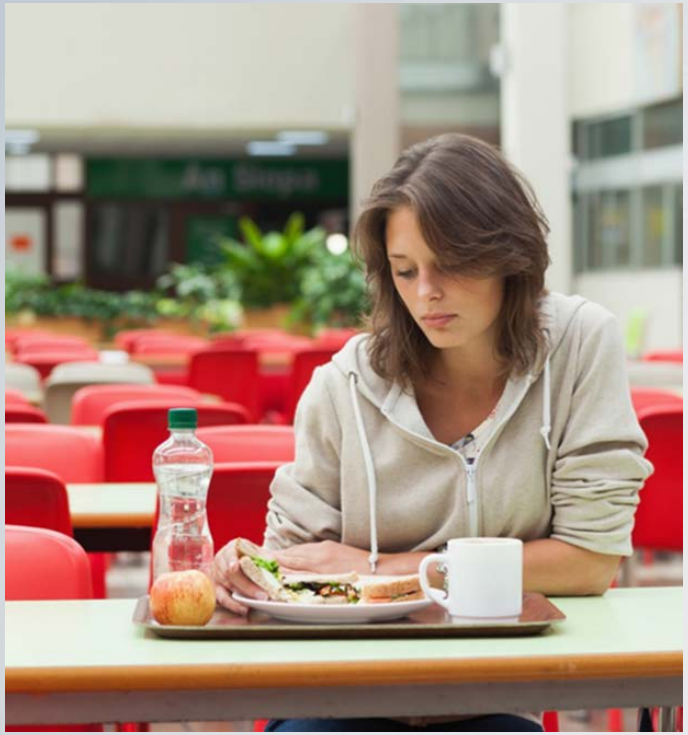
- Met primary study objective of preventing activation of IFN-gamma producing gliadin-specific cells during gluten challenge (GC)
- Associated with a trend towards a reduction in the GC induced villous height:crypt depth ratio deterioration
- Reduced circulating, gut-homing, CD4+ and CD8+ cells during GC
- First clinical trial to demonstrate induction of antigen specific immune tolerance in any autoimmune disease

Kelly C, Murray J, Leffler D, Bledsoe A, Smithson G, Podojil J, First R, Morris A, Boyn M, Elhofy A, Wu T, and Miller S: Cour- Takeda

Frontiers of Celiac Disease

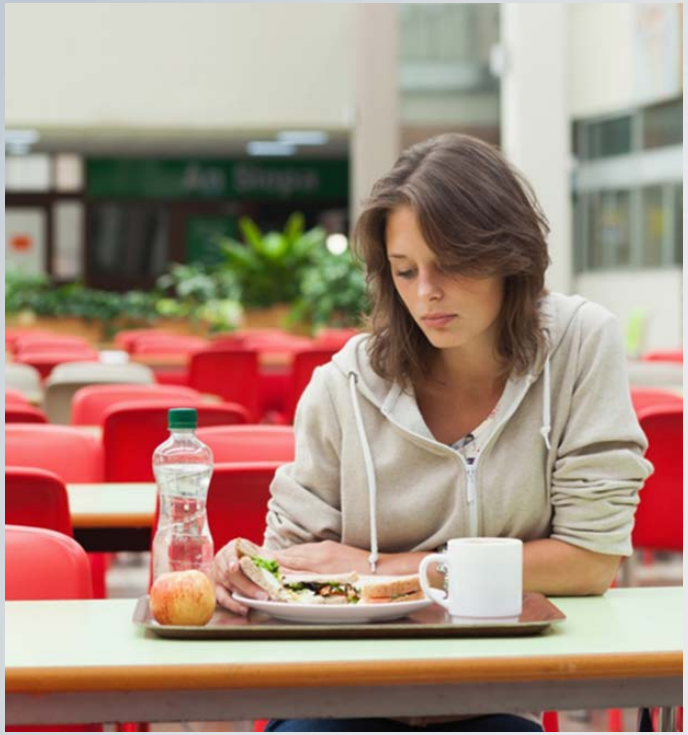


Frontiers of Celiac Disease



Celiac disease is a widely misunderstood genetically based autoimmune disorder affecting more than 1% of the world population

Frontiers of Celiac Disease



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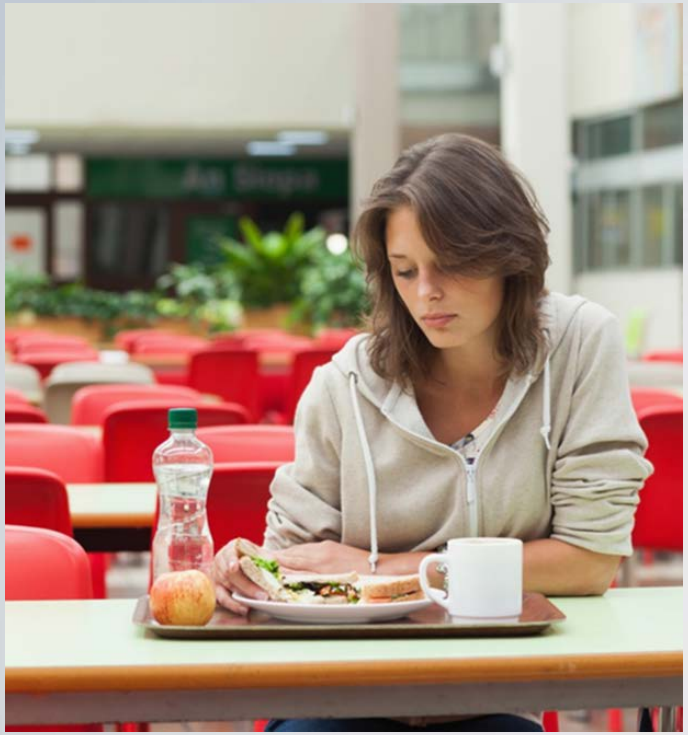
Frontiers of Celiac Disease



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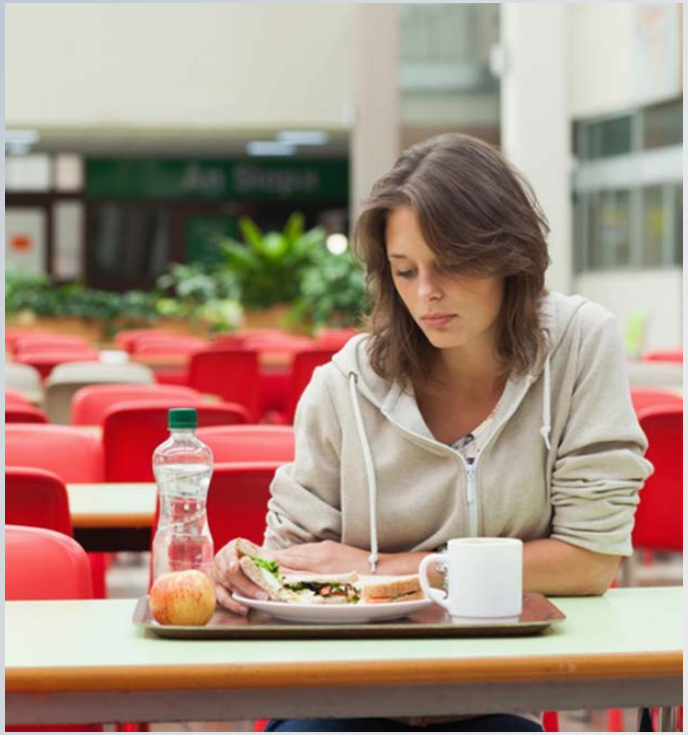
Frontiers of Celiac Disease



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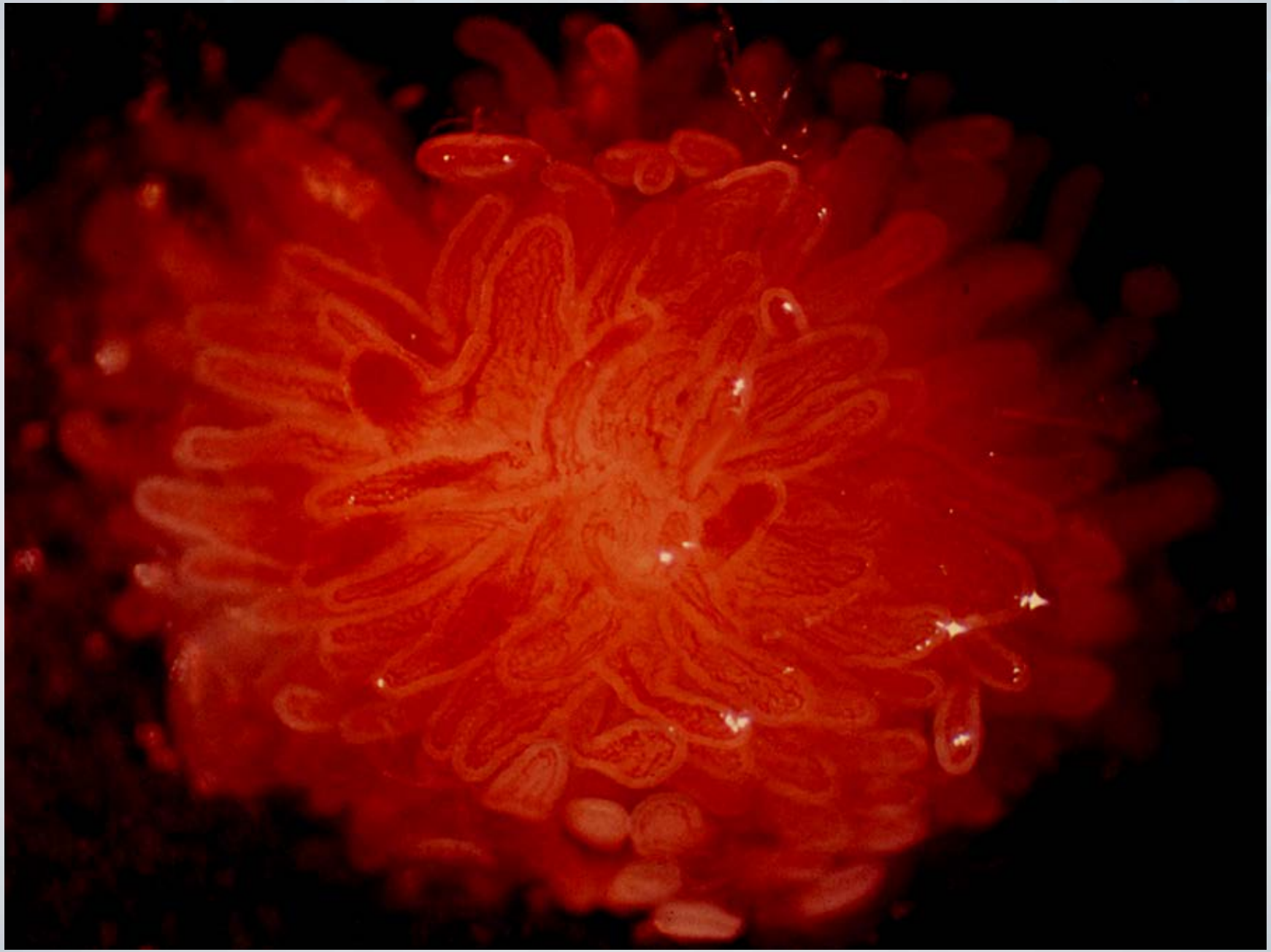
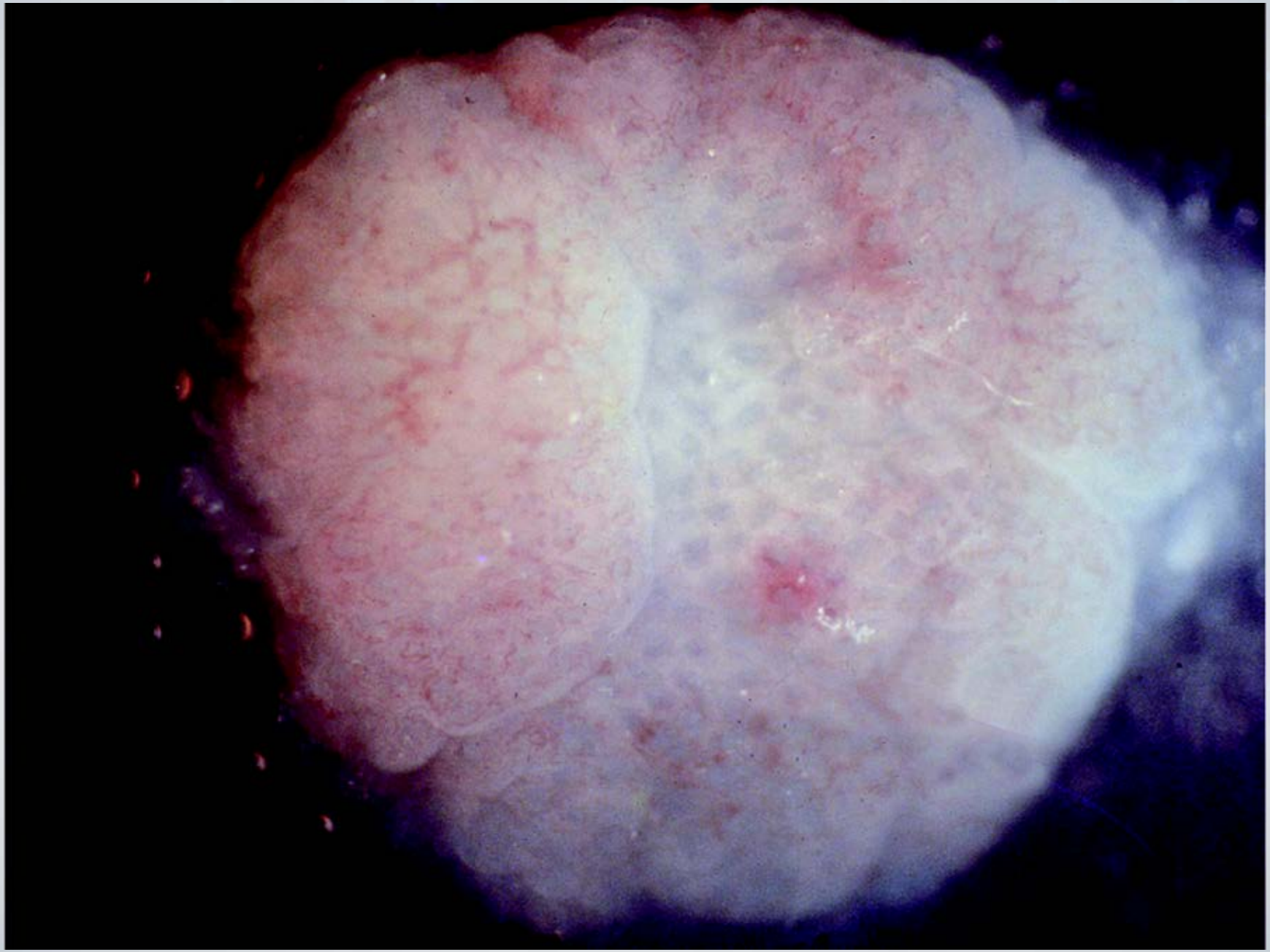
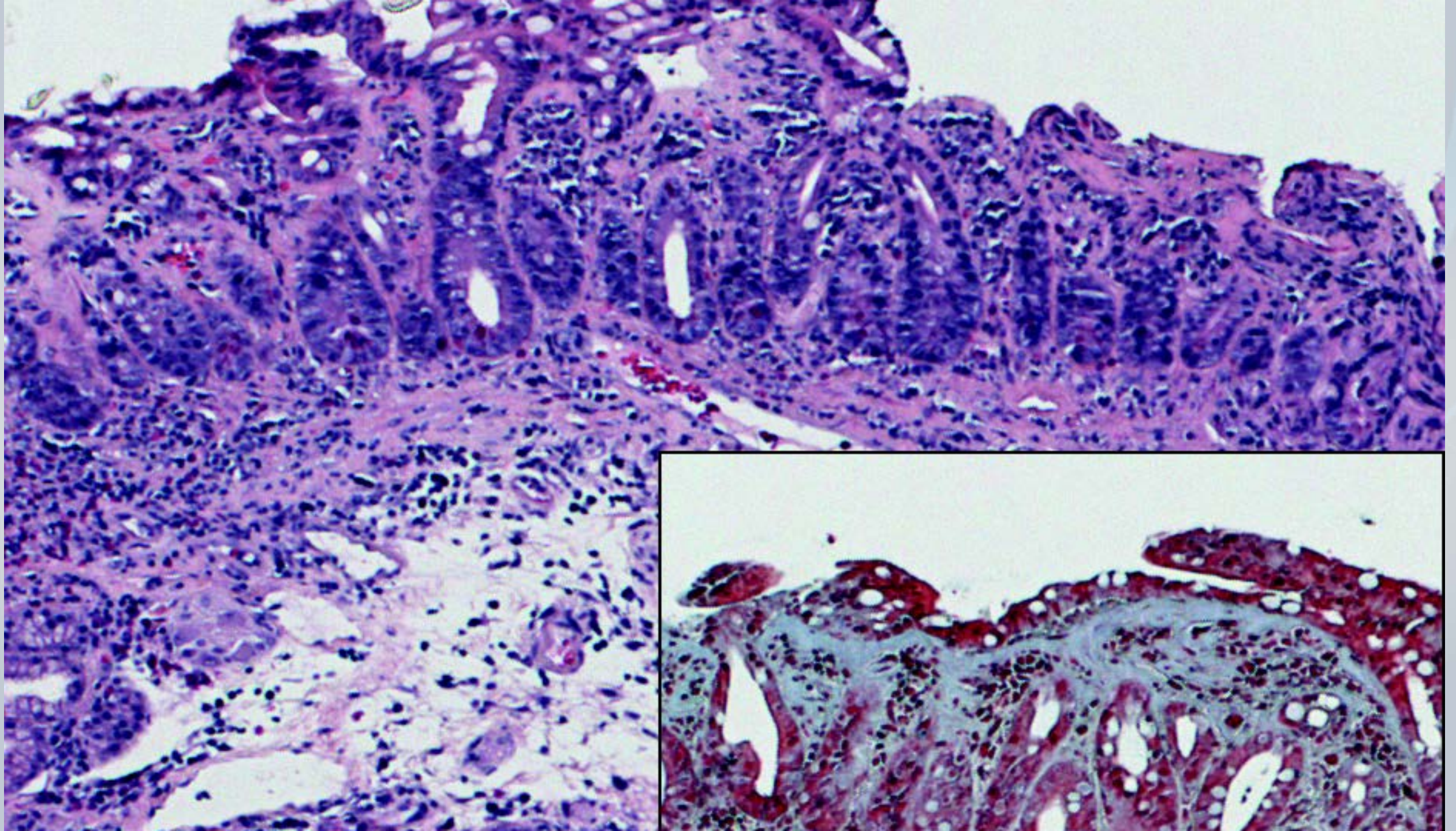
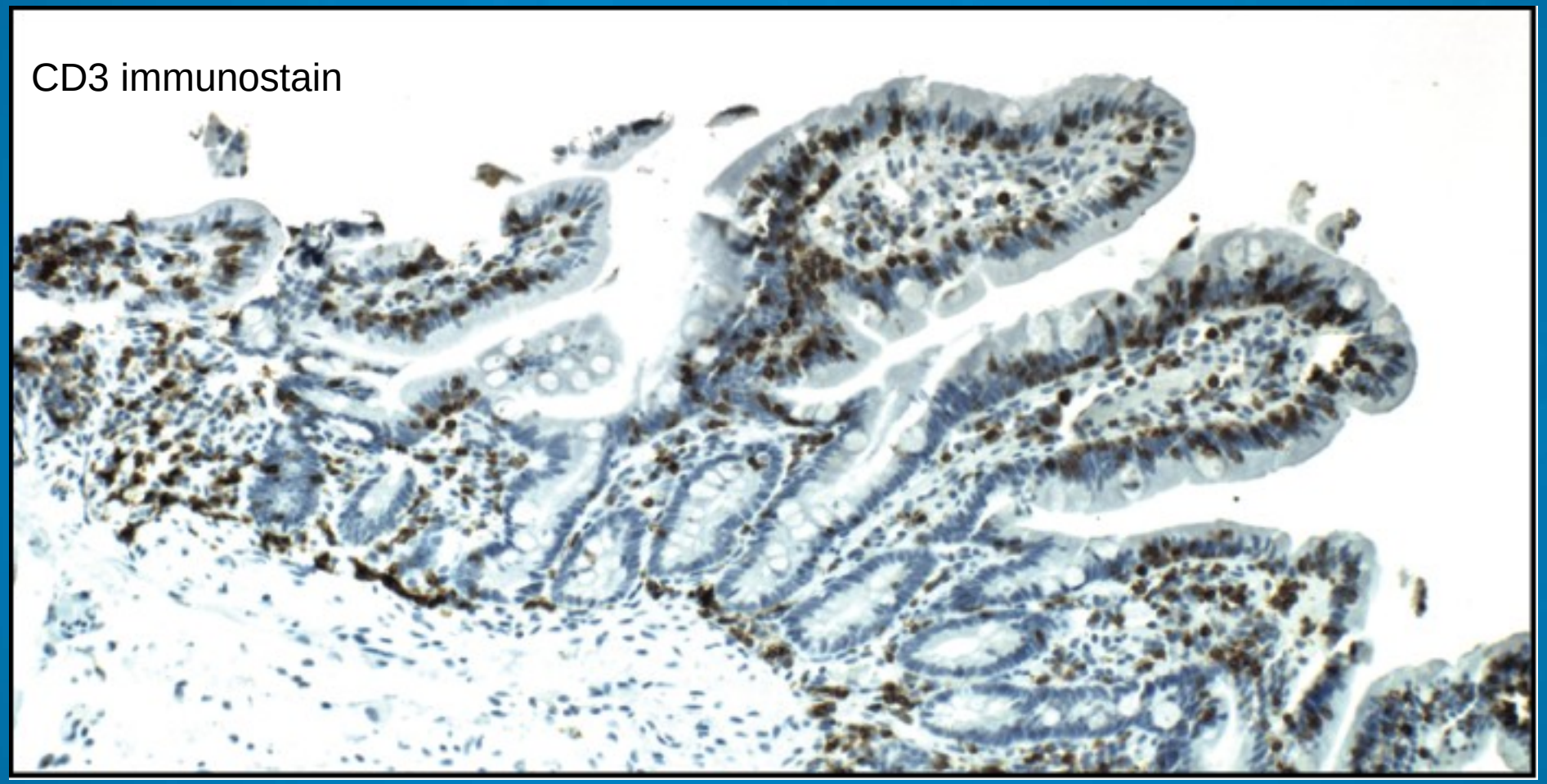
Pathologists are a key part of this evolving story.

The role of biopsy interpretation is likely to increase as patients get more follow up biopsies, participate in clinical trials, and in the pursuit of biomarker development.









Be careful not to count IELs really in lamina propria