Laboratory diagnostics of inflammation and non specific inflammatory diseases of gastrointestinal tract

Plan

- Inflammation
  - Classic parameter of inflammation (leucocytosis, ESR, CRP)
  - Special parameter of inflammation (IL-6), Прокальцитонин (PCT)
  - Immunoglobulins and paraproteins in inflammation
- Autoimmun gastritis
- Coeliac disease
- Non specific inflammatory bowel diseases (Crohn disease and non specific ulcerative colitis)

INFLAMMATION

- Inflammation is response of the body to **homeostasis disorders** of various origins: a **pathogenic microorganisms** (infection), **tissue damage** of non-infectious origin, caused by mechanical factors (injury - trauma), burns, poisons, metabolic stress, tissue hypoxia.
- Inflammatory reactions also occur in **allergies and autoimmune diseases.**

Classic (basic) markers of inflammation

- Leukocytosis
- ESR
- CRP

Erythrocytes Sedimentation Rate (females 6-20 mm/h, males 3-10 mm/h, in patients >50years 50% more)

Up to 50mm/h	50-100 mm/h	>100 mm/h	Decreasing
• Error of determination (heat influence, too much Citrat, less blood in the sample-tube) • Anemia • Hypertriglyceridemia • Contraception, • pre menstruation, pregnancy • Tuberculosis, brucellosis • Tumors	• Infection (mainly bacterial) • Tumors with metastasis? Leucosis • Hemolytic anemias • Chronic diseases of liver • Nephrotic syndrome Chronic renal insufficiency • Tissue necrosis • Rheumatoid arthritis, collagenoses, vasculitis	• Infection • Sepsis • Peritonitis • Rheumatoid fever • Myelom disease • Rheumatic myalgia • Vasculitis	• Error of determination (cold influence, less Citrat) • Polycythemia vera • Abnormally shaped erythrocytes (sickle cell anemia) • Dehydration

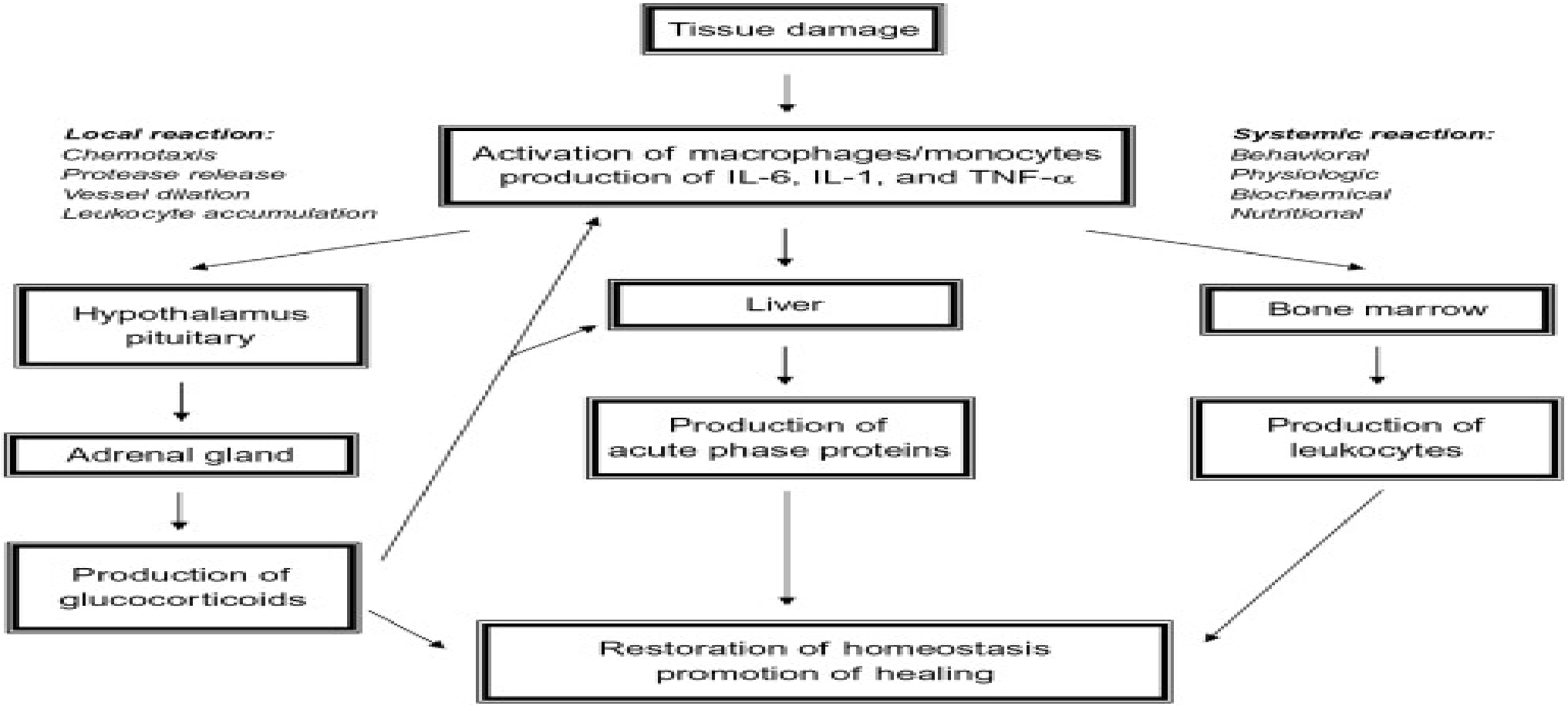
C-reactive protein (<5 mg/l)

- **CRP** – an acute phase protein of inflammation that, as a result of the action of cytokines IL-6 and IL-1β, is formed and released from liver cells. CRP increases in inflammation, tissue necrosis. CRP activates also platelets, white blood cells, and endothelial cells.
- CRP increases during 6-10 hours after beginning of disease (ESR increases later).
- CRP doesn’t depend on number of erythrocytes, it’s shape and other ”erythrocytes” fastors.

Special markers of inflammation

- IL-6 (increase 2-4 hours after beginning of inflammation)
- TNF-α
- Procalcitonin

Production of special markers of inflammation



PROCALCITONIN (PCT)

- PCT is a complex glycoprotein, a precursor to the thyroid hormone calcitonin, as well as an indicator of the systemic inflammatory process in the body, is superior in sensitivity and specificity to inflammatory markers - CRP and IL-6. In the presence of systemic inflammation, PCT is formed not only in the thyroid gland, but also in RES cells (reticulo-endothelial system). The main activators of these processes are TNF-α, IL-6, as well as LPS (liposaccharides) of Gram-negative bacteria.
- Normally, the PCT content in the blood is extremely low. The amount of procalcitonin in a healthy person is less than 0.5µg/l. In bacterial infections, PCT levels increase significantly.
- It’s determinated in department for intensive therapy (more expensive than CRP and ESR)

PROCALCITONIN (PCT)

N	< 0,5 µg/l
A	Indications for definition: differentiation of fever of bacterial and non-bacterial origin
↑	**At > 5µg/l indicates sepsis; differentiation of infection of viral (normal or slightly increased) and bacterial (significant increase) origin; indicates multiple organ failure**

Causes of procalcitonin increase

1. increased procalcitonin levels associated with infections: sepsis with confirmed or unconfirmed bacterial infection, sepsis-related conditions such as acute pancreatitis, systemic infections that can occur in pneumonia or acute pyelonephritis, systemic viremias, fungal infections, severe malaria;

- 2. increased procalcitonin levels not associated with infections: burns, injuries, sunstroke, heatstroke;
- 3. increased procalcitonin levels associated with diseases of respiratory system: aspiration and inhalation pneumonia, adult respiratory distress syndrome (ARDS), pulmonary neuroendocrine hyperplasia occurring in chronic obstructive pulmonary disease (COPD) or chronic bronchitis associated with smoking;
- 4. increased procalcitonin associated with malignant tumors: medullary thyroid cancer (C-cell thyroid cancer), small cell lung cancer, non-small cell lung cancer, carcinoid tumor, other neuroendocrine tumors (pheochromocytoma, insulinoma), breast cancer;
- 5. severe chronic renal failure.

Proteins in inflammatory diseases

- Immunoglobulins
- Paraproteins

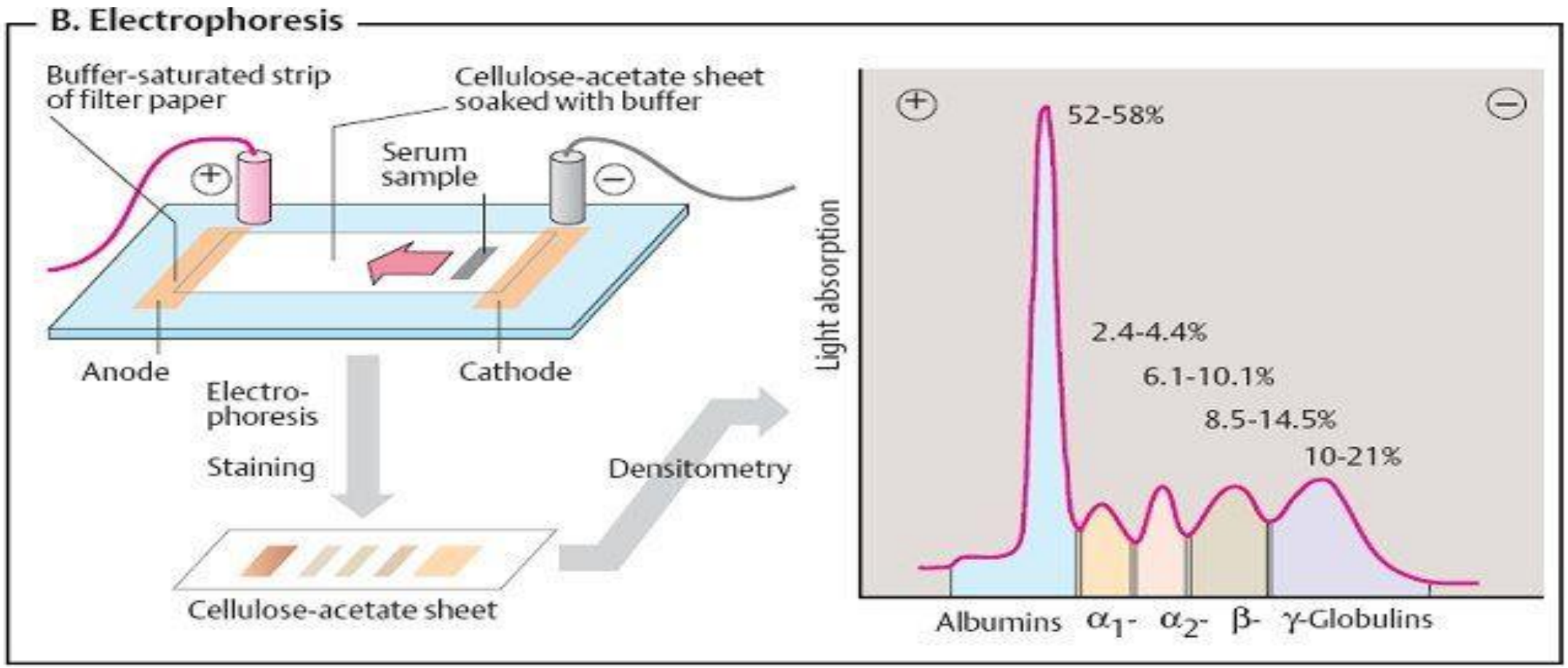
Indications for determination of Ig and paraproteins

- Clinical signs of immunodeficiency, possibly due to decreased γ-globulin levels in serum electrophoresis;
- Detection of intrauterine infections in newborns;
- Increase in β- or γ-globulins in serum electrophoresis;
- M-gradient in serum electrophoresis;
- Clinical symptoms (bone pain, rheumatic complaints, anemia, especially in elderly patients) associated with increased ESR, proteinuria;
- Renal failure of unknown origin.

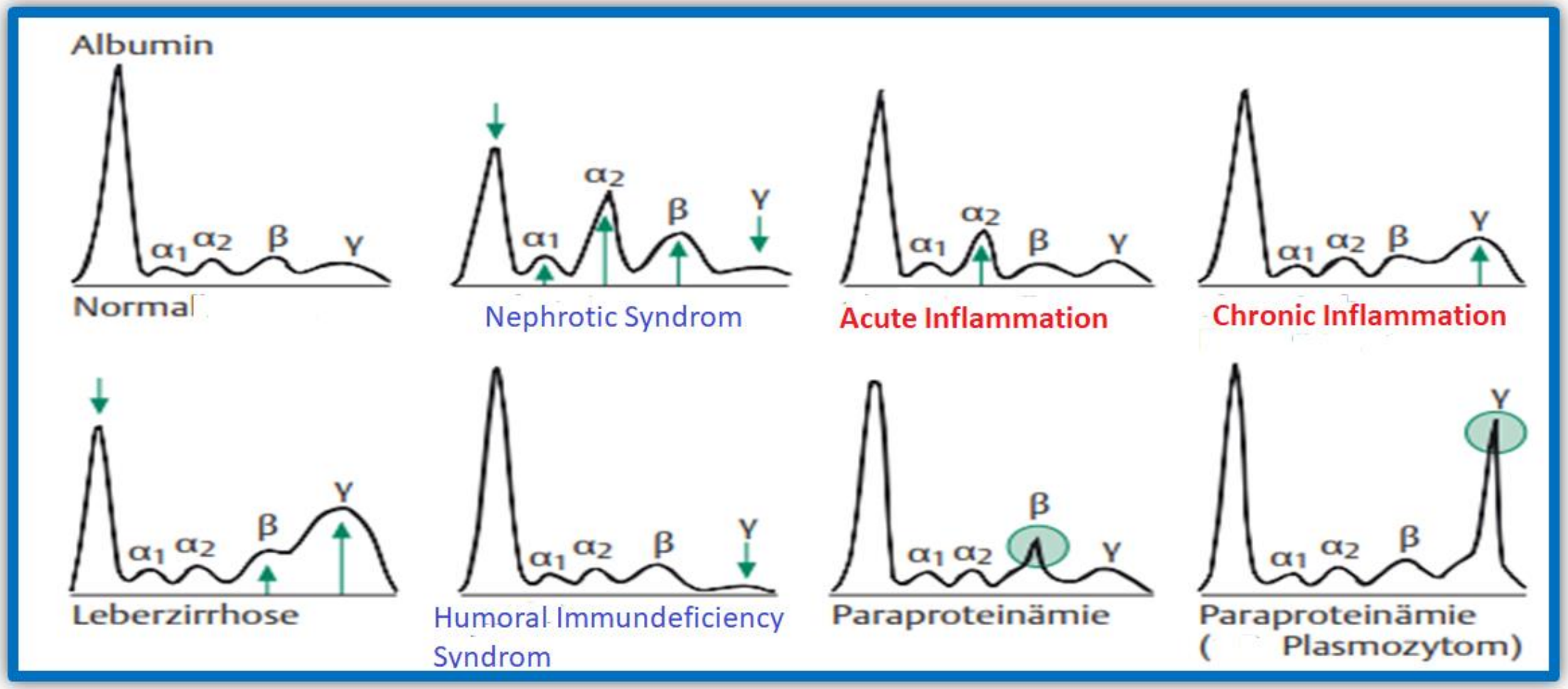
Immunoglobulins are also determined for the diagnosis of hypersensitivity reactions

- Allergies (IgE-dependent allergic reactions, IgE-independent allergic reactions of type III and IV)
- Autoantibodies (Rheumatoid factor RF, Autoantibodies against citrullinated peptides/proteins-ACPA, Antinuclear antibodies - ANA, Antineutrophil cytoplasmic antibodies ANCA, Antimitochondrial antibodies AMA, Antibodies to smooth muscle cells - ASMA)

Proteins electrophoresis

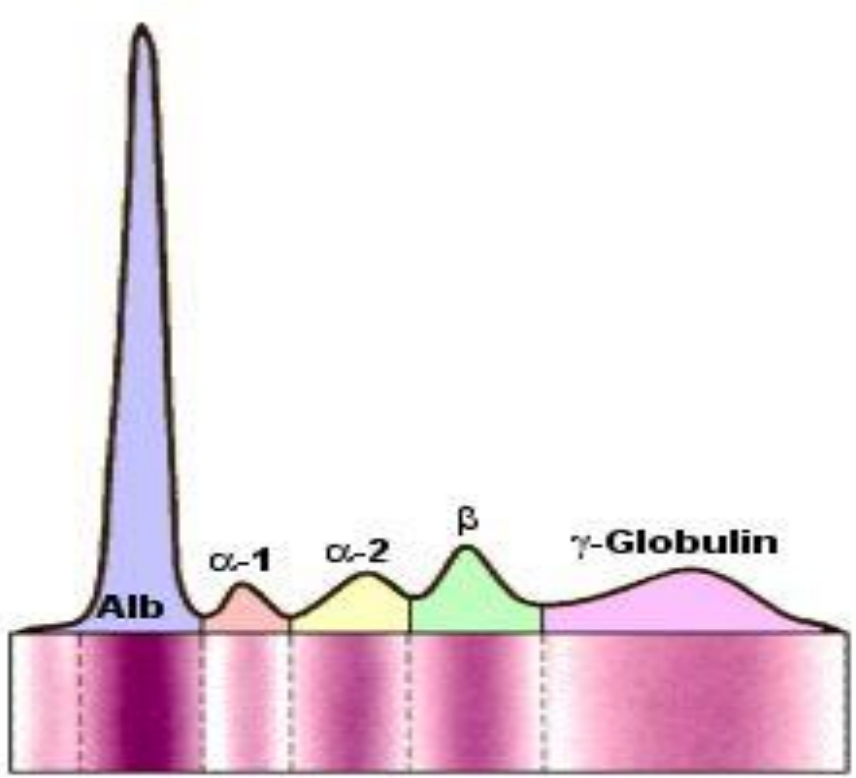


Pathological changes in electrophoresis of the blood plasma



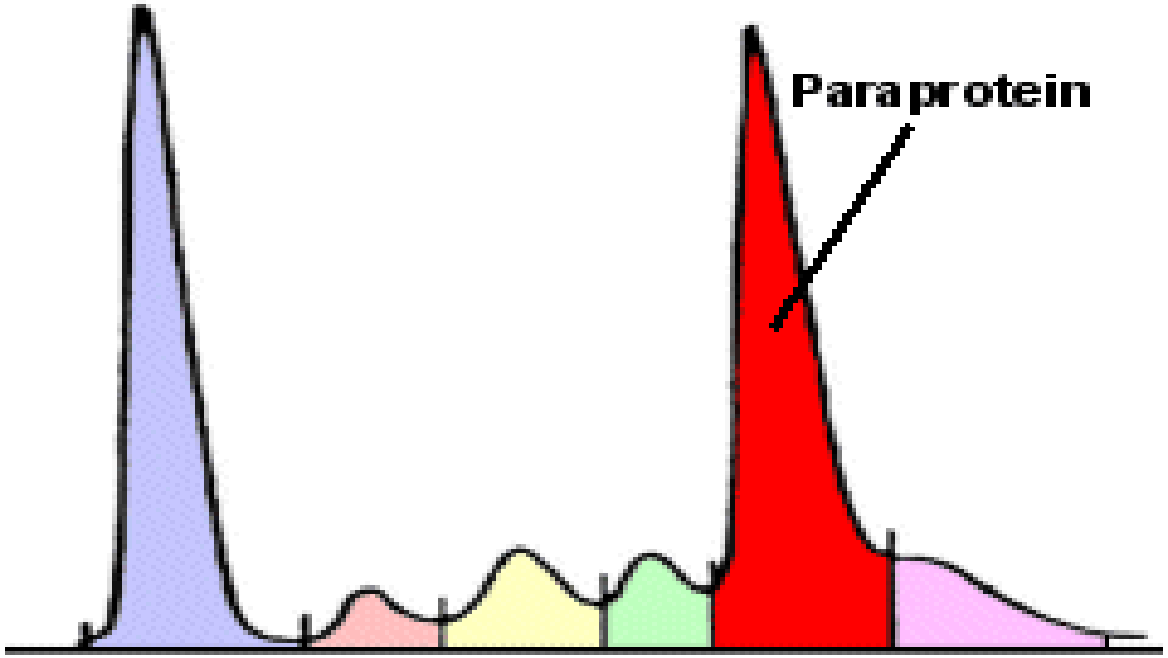
Proteins electrophoresis

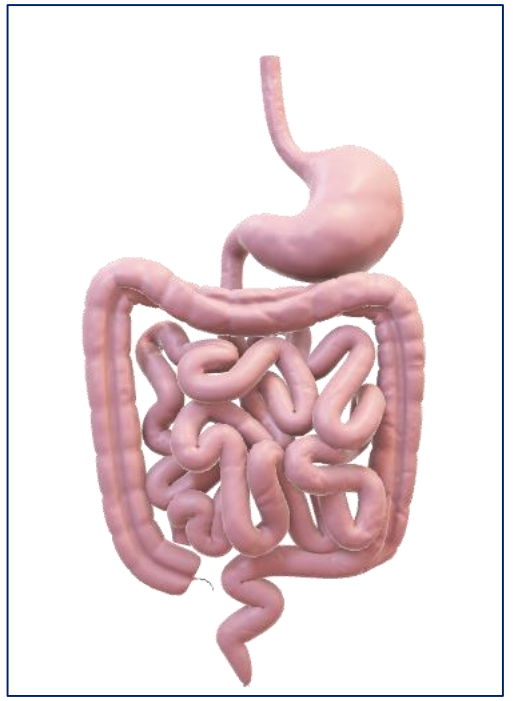
Norma



Myeloma

(M-gammopathy with M-gradient in electrophoresis)





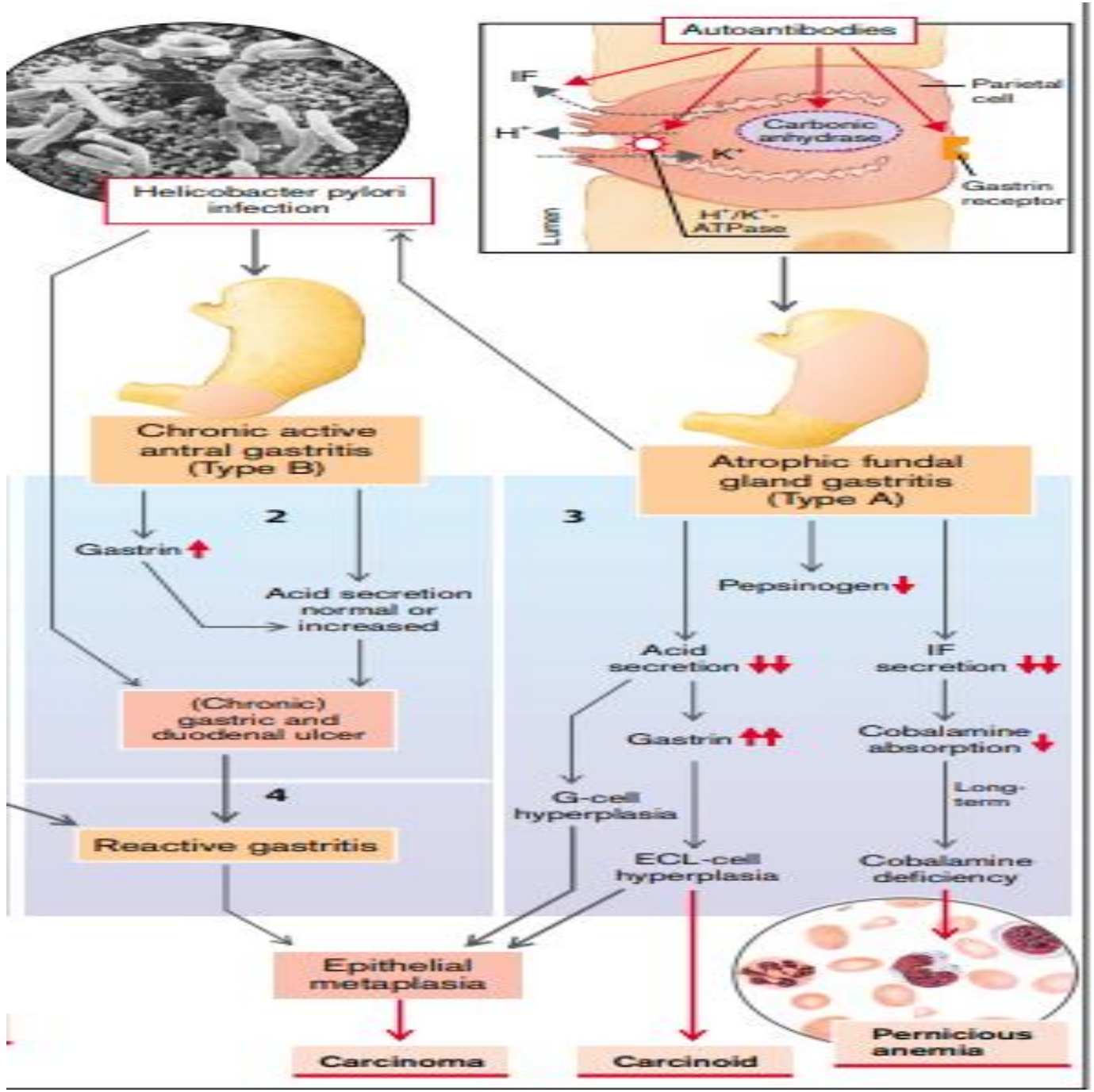
Non specific inflammatory diseases of digestive tract

Autoimmune gastritis

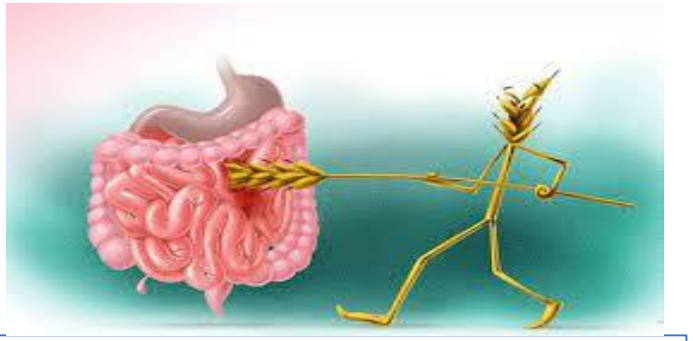
- Autoimmune gastritis (type A of chronic gastritis) results from the production of autoantibodies (mainly immunoglobulin G, infiltrates of plasma cells, and B lymphocytes) to the parts and products of gastric parietal cells, including antibodies against the acidproducing enzyme H^+,K^+-ATPase, gastrin receptor, and Castle's intrinsic factor.

Autoimmune gastritis

- presence of antibodies to parietal cells and IF in serum and gastric juice;
- decrease of pepsinogen concentration in blood serum;
- hyperplasia of endocrine cells in the antrum of the stomach;
- vitamin B12 deficiency;
- impaired secretion of hydrochloric acid in the stomach (achlorhydria).

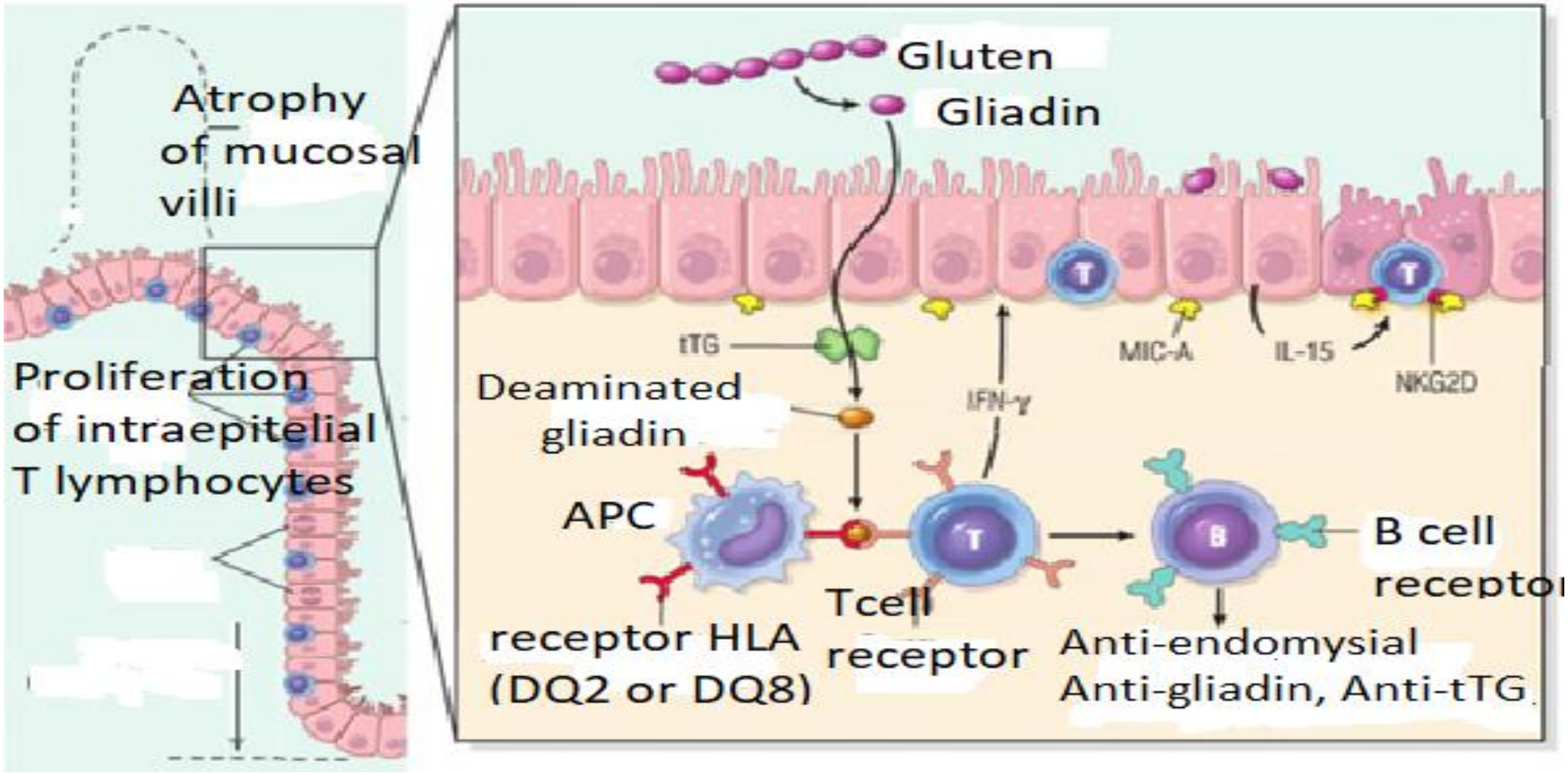

Helicobacter pylori infection
Autoantibodies
IF
H+
K+
Parietal cell
Carbonic anhydrase
Gastrin receptor
Lumen
H+/K+-ATPase
Chronic active antral gastritis (Type B)
Atrophic fundal gland gastritis (Type A)
2
3
Gastrin
Acid secretion normal or increased
Pepsinogen
Acid secretion
IF secretion
(Chronic) gastric and duodenal ulcer
Gastrin
Cobalamine absorption
4
G-cell hyperplasia
Long-term
Reactive gastritis
ECL-cell hyperplasia
Cobalamine deficiency
Epithelial metaplasia
Pernicious anemia
Carcinoma
Carcinoid

CELIAC DISEASE



- *Celiac disease*, also known as *non-tropical sprue or gluten-sensitive enteropathy*, is an immune-mediated enteropathy caused by eating gluten-containing cereals (wheat, rye, or barley).
- As a result of destruction of enterocytes by CTL (cytotoxic T-lymphocytes) in intestine develops ***immune inflammation***, which leads to malabsorption syndrome.
- Celiac disease develops only in genetically predisposed people (HLA-DQ2 genes, HLA-DQ8).

CELIAC DISEASE. Pathogenesis.



Pathogenesis of celiac disease

Protease

- Gluten – Gliadin – expression of IL-15 by epitheliocytes – activation and proliferation of intraepithelial T-lymphocytes CD8+ - binding NKG2D+MIC-A of enterocytes – damage of epithelium by CTL

tTG

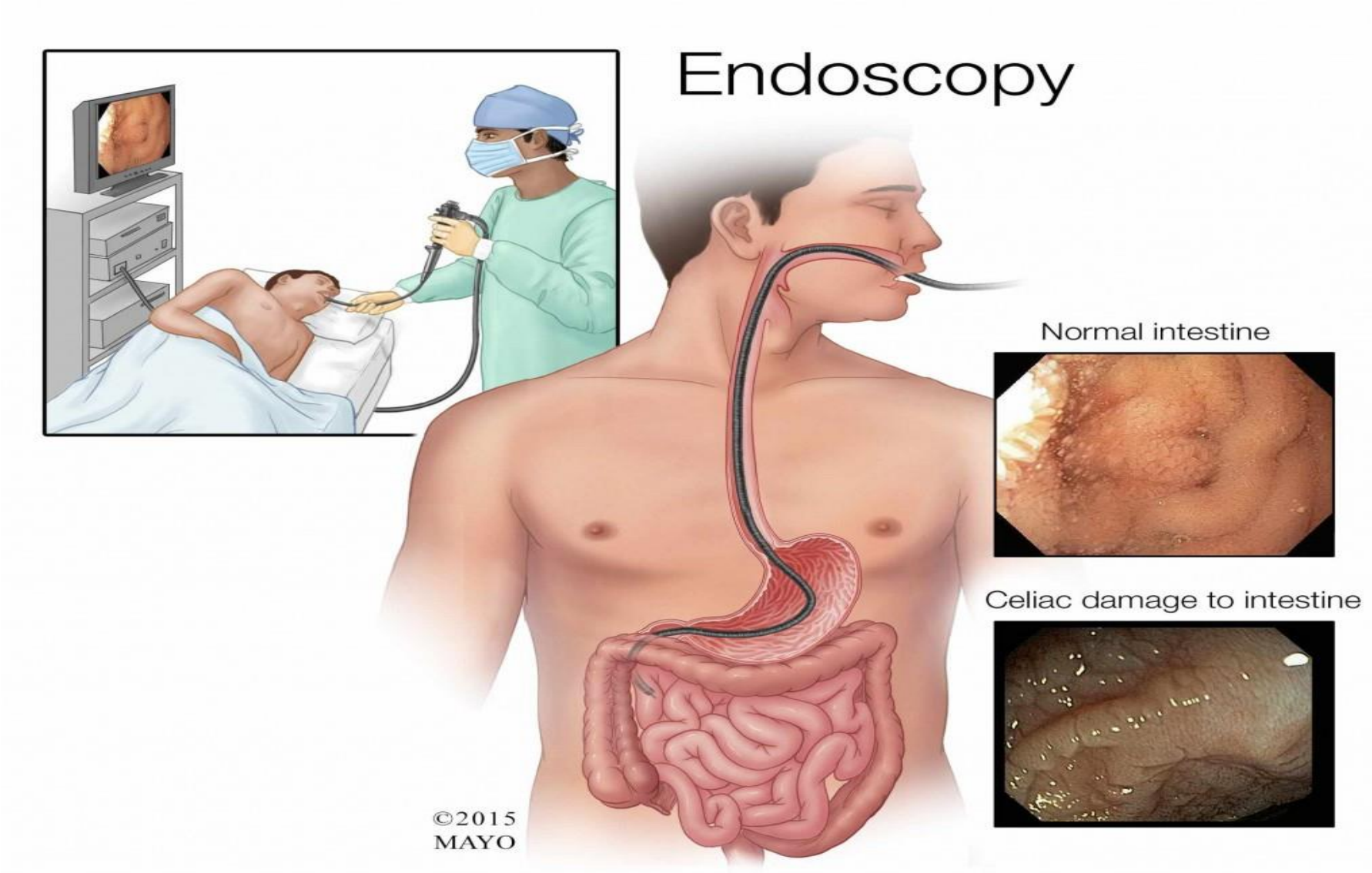
- Gliadin - deaminated gliadin+HLA-DQ2 (95%) or -DQ8 (2%) on APC (epitheliocyte) – presentation of gliadin to Th CD4+ – T-lymphocytes release cytokins, which send the signal to B-lymphocytes. As a result B-lymphocytes produce antibodies against gliadin, endomysium and tTG.


Celiac Disease
Normal
Damaged

Serological diagnostics of CD

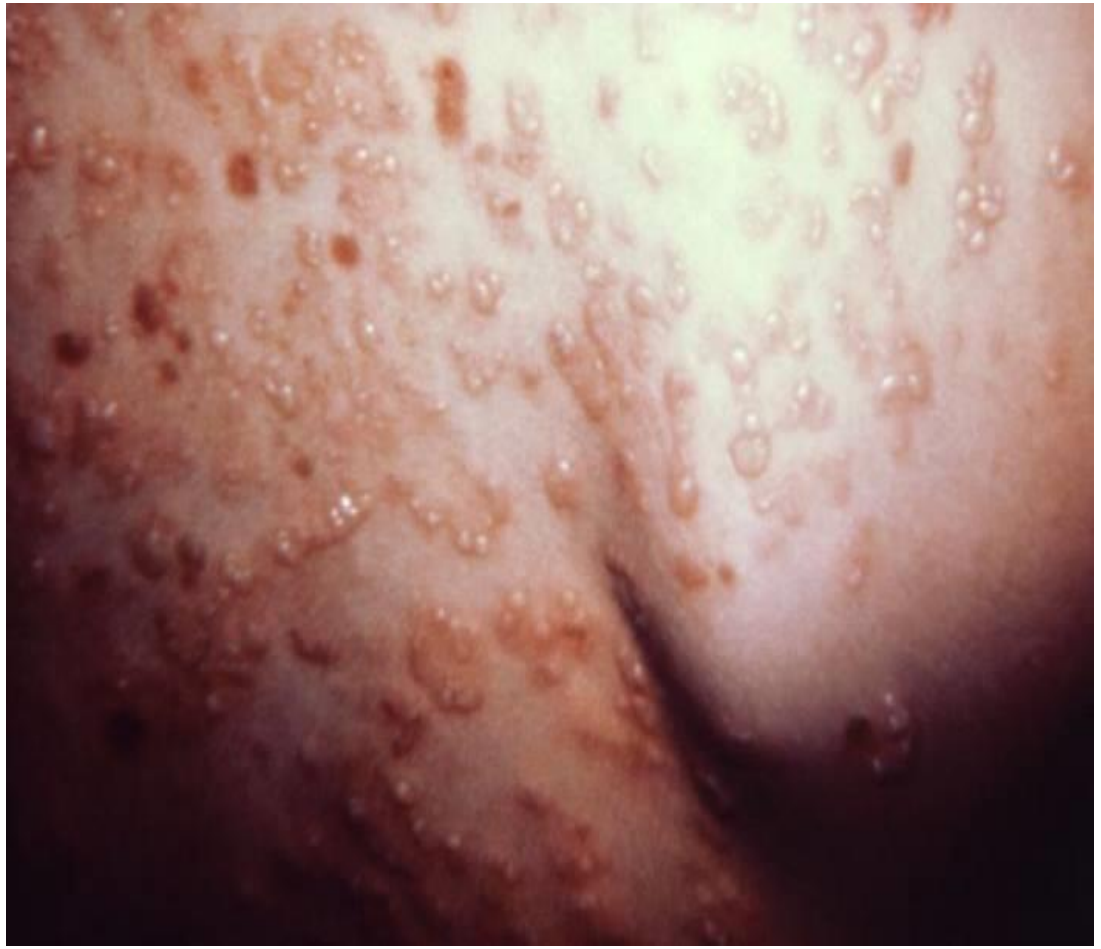
- **IgA endomysial antibodies (IgA EMA);** moderately sensitive and highly specific in untreated (active) celiac disease). The test results are assessed simply - as positive or negative, since even low titers of serum IgA endomysial antibodies are **specific** for celiac disease.
- **IgA tissue transglutaminase antibodies (IgA tTG).**Anti-tTG antibodies are highly sensitive and specific for the diagnosis of celiac disease.
- **IgA antiglyadine antibodies (IgA AGA).** Although these tests demonstrate moderate sensitivity and specificity (especially IgA), their value as an indicator of disease in the general population is relatively low.
- **IgG antiglyadine antibodies (Ig G AGA).** AGA tests are not currently recommended for use due to their low sensitivity and specificity.

Endoskopic investigation in CD



CD+Dermatitis herpetiformis

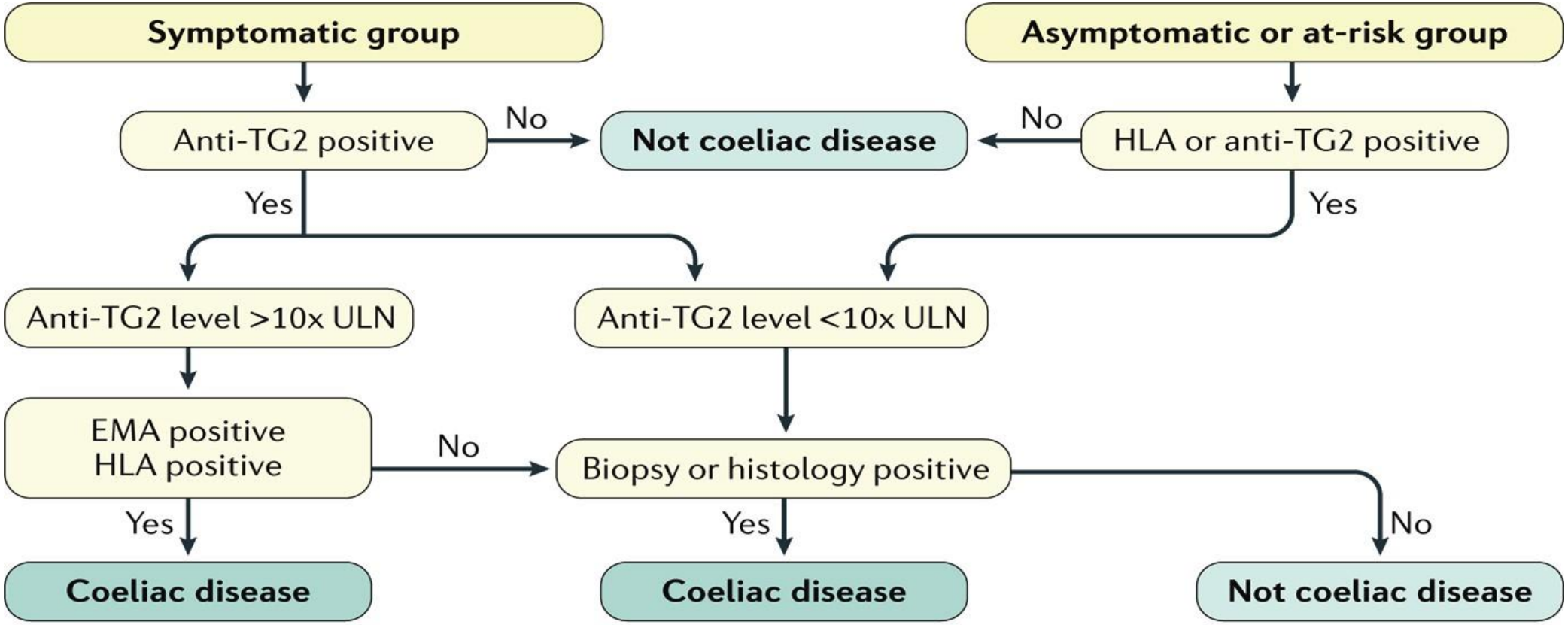
- Dermatitis herpetiformis is associated with IgA antibodies against epidermal transglutaminase (eTG), which does not occur in celiac disease.
- Patients with herpetiform dermatitis with abnormal blood results usually undergo a biopsy of the small intestine to confirm gluten-sensitive enteropathy.



Specific tests for herpetiform dermatitis with CD:

- IgA anti-endomysial antibodies
- IgA, tTG antibodies
- IgA antibodies to epidermal transglutaminase *, eTG (if present)
- IgA and IgG antibodies to deaminated gliadin peptides, dGP
- Total IgA level
- HLA haplotype, a set of DNA variations, testing can reveal HLA-DQ2 (90-95% of cases) or HLA-DQ8. This is present in almost all patients with dermatitis herpetiformis (and celiac disease).
- Complete blood count, liver function tests and serum calcium levels
- Iron, zinc, vitamin B12 and folic acid
- Thyroid function tests.

Diagnostic algorythmus of CD



Nature Reviews | Gastroenterology & Hepatology

GOLD STANDART in DIAGNOSIS of CD

Intestinal biopsy with serological testing

- American College of Gastroenterology: Clinical guidelines for the diagnosis and management of celiac disease
- DGIM

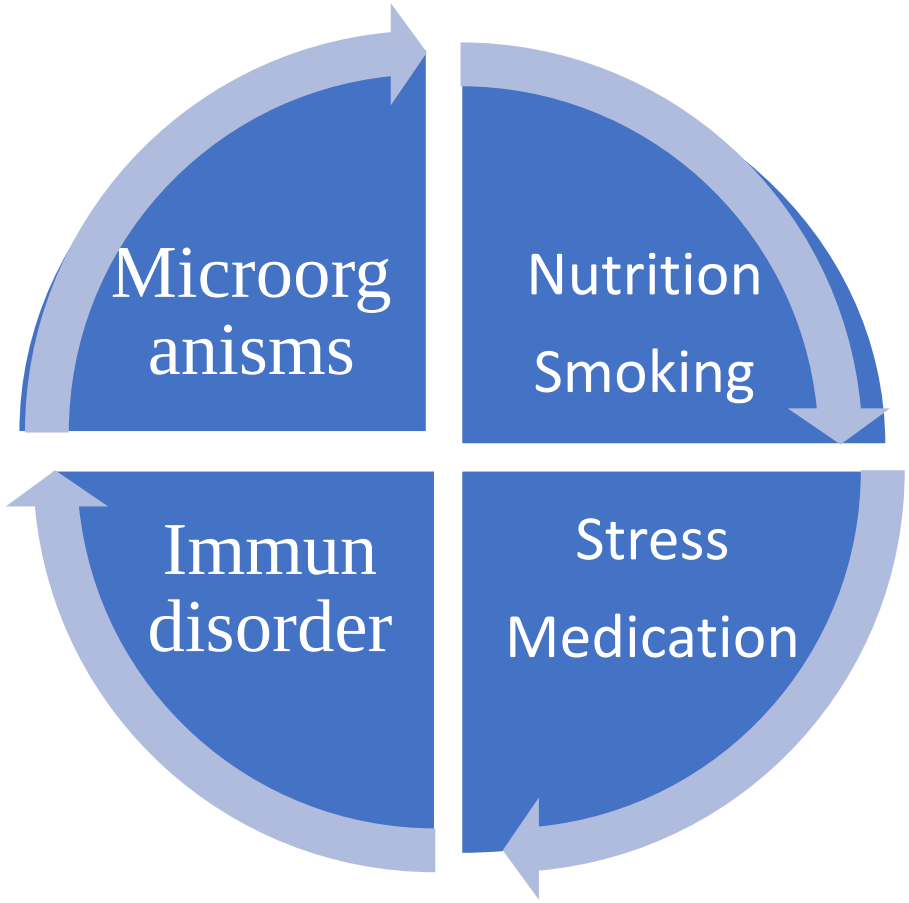
Individuals with celiac disease have a higher risk of developing malignancies:

- **enteropathy-associated T-cell lymphoma,**
- **small intestine adenocarcinoma…**

Nonspecific inflammatory bowel disease (IBD)

- IBD is a chronic disease resulting from inadequate activation of the immune defense components of the intestinal mucosa. IBD is represented by two diseases - Crohn's disease (CD) and nonspecific ulcerative colitis (UC).

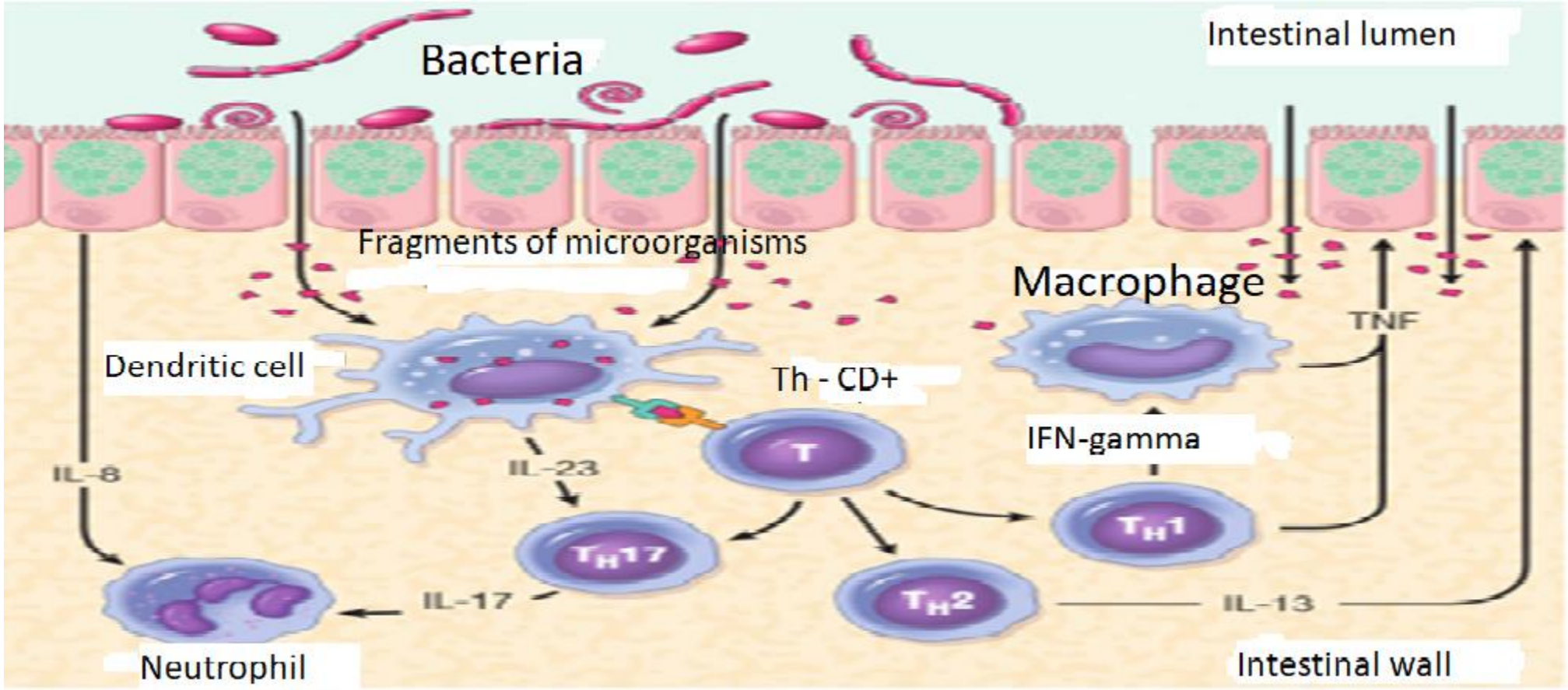
Triggers of CD and UC



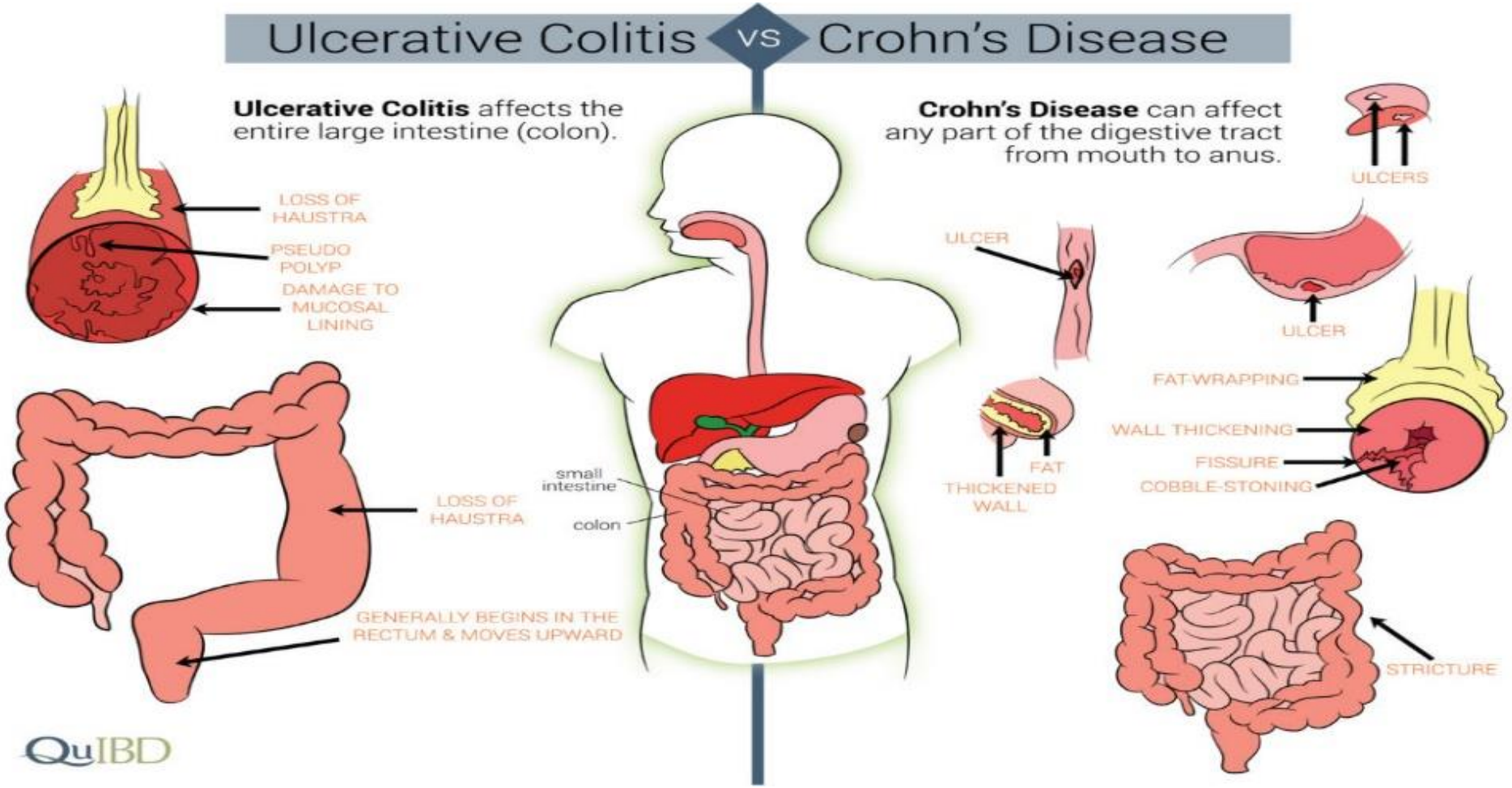
Pathogenic Factors in IBD

- Genetic factors
- Immune response of intestinal mucosa in IBD
- Defects in epitheliocytes
- Intestinal microflora

PATHOGENESIS of IBD



Nonspecific inflammatory bowel diseases



Differences between CD and UC

Feature	Crohn disease	Ulcerative colitis
Bowel region	Ileum±Colon	Colon
Stricture	Early	Late/rare
Wall appearance	Thickened	Thin
Dilation	No	Yes
Pseudopolypen	None	Marked
Ulcer	Deep, linear	Superficial
Granulomas	Yes	No
Fistulas	Yes	No
Fat/vitamin malabsorbtion	Yes	No
Malignant potential	Yes	Yes
Response to surgery	Poor	Good

Extraintestinal signs of CD

Рисунок 2. Афтозные язвы ротовой полости при болезни Крона (Forbes et al., 2005)

Diagnosis of IBD

- Clinical diagnosis (diarrhea, with mucus, blood in feces – hematochezia, fever, abdominal pain)
- Labor (monocytosis, anemia, thrombocytosis, classic and special markers of inflammation – ESR, CRP, IL-6, TNF, PCT), immunological analysis (pANCA, anti-saccharomyces cerevisiae antibodies -ASCA), calprotectin in feces
- Instrumental (Colonoskopy, Ultrasoud, MRT, CT…)
- Hystological

Endoskopy in CD

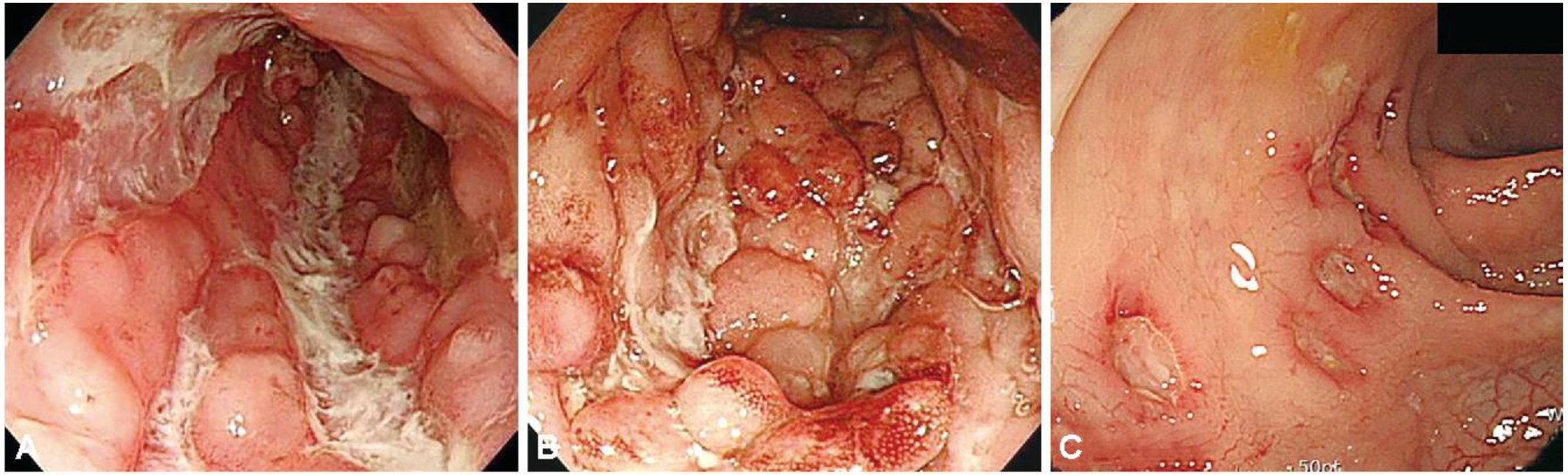


Fig. 2. Typical endoscopic features of Crohn's disease. (A) Longitudinal ulcers, (B) cobblestone appearance, (C) aphthous ulcers showing longitudinal array.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