Failure of immune tolerance: gastrointestinal tract immunity defects

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Outline

- Basic principals of immune tolerance
- Case report
- Mucosal immunity and mucosal tolerance

Immune tolerance

- Protects the integrity of organism and its function by eliminating the reaction against “safe” antigens

- Is antigen specific and is a result of the interaction and recognition of the antigen by immune system

What antigens are tolerated?

- Self antigens (autologous antigens)

- Common environmental antigens
  - food antigens
  - gut microbial flora antigens (comensal microbiota antigens)
  - antigenic components of dust, pollen etc.

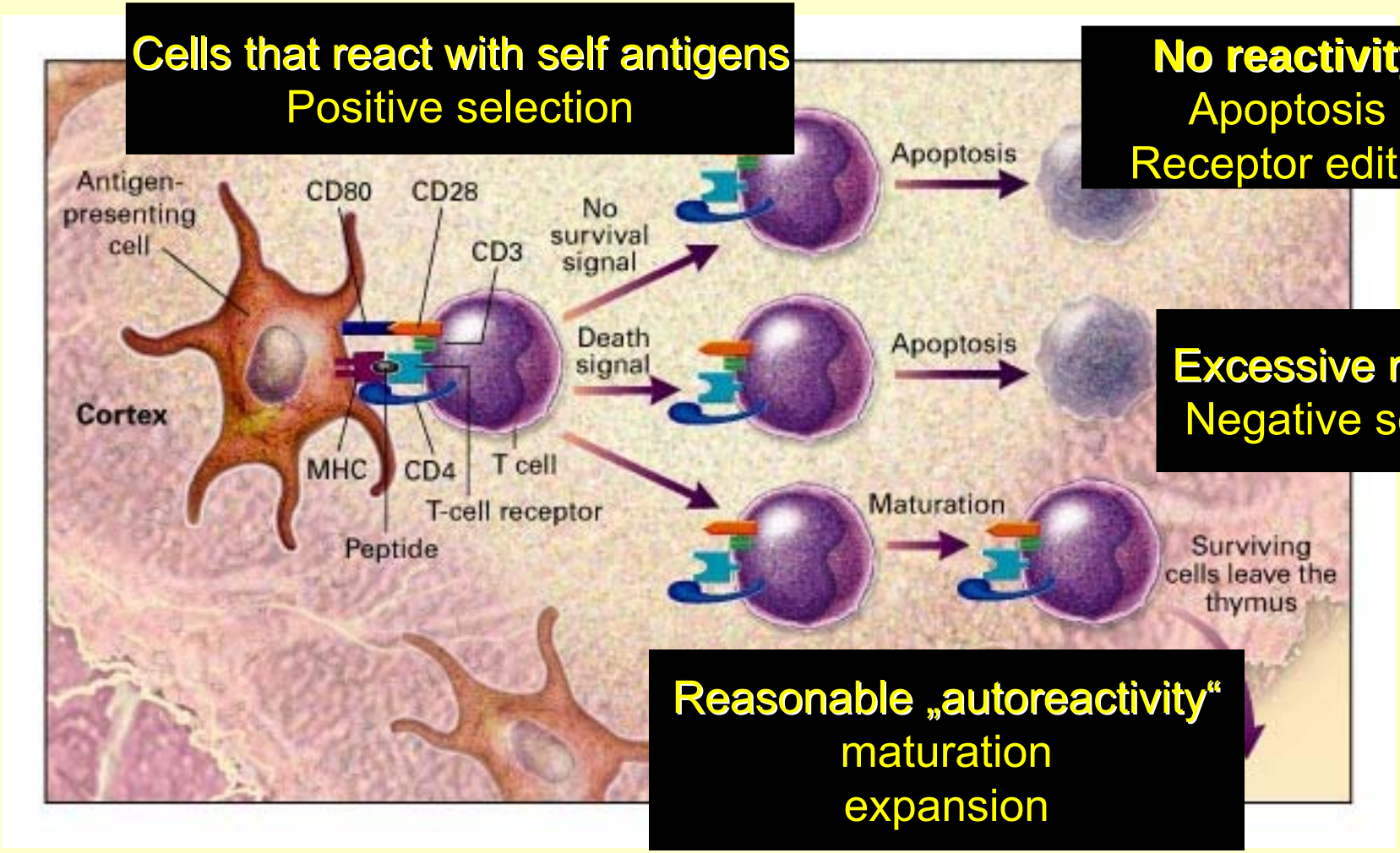
How and where is the tolerance of self antigens induced?

- Central mechanism
  - Thymus (T - cells) / Bone marrow (B - cells)
- Peripheral mechanism
  - Lymph nodes, lymphoid tissues

- **Antigent presenting cells**, antigen presenting molecules, costimulatory molecules, cytokines, cytokine receptors, adhesion molecules, **lymphocytes** and other cells

Central Mechanism of Immune Tolerance Thymus

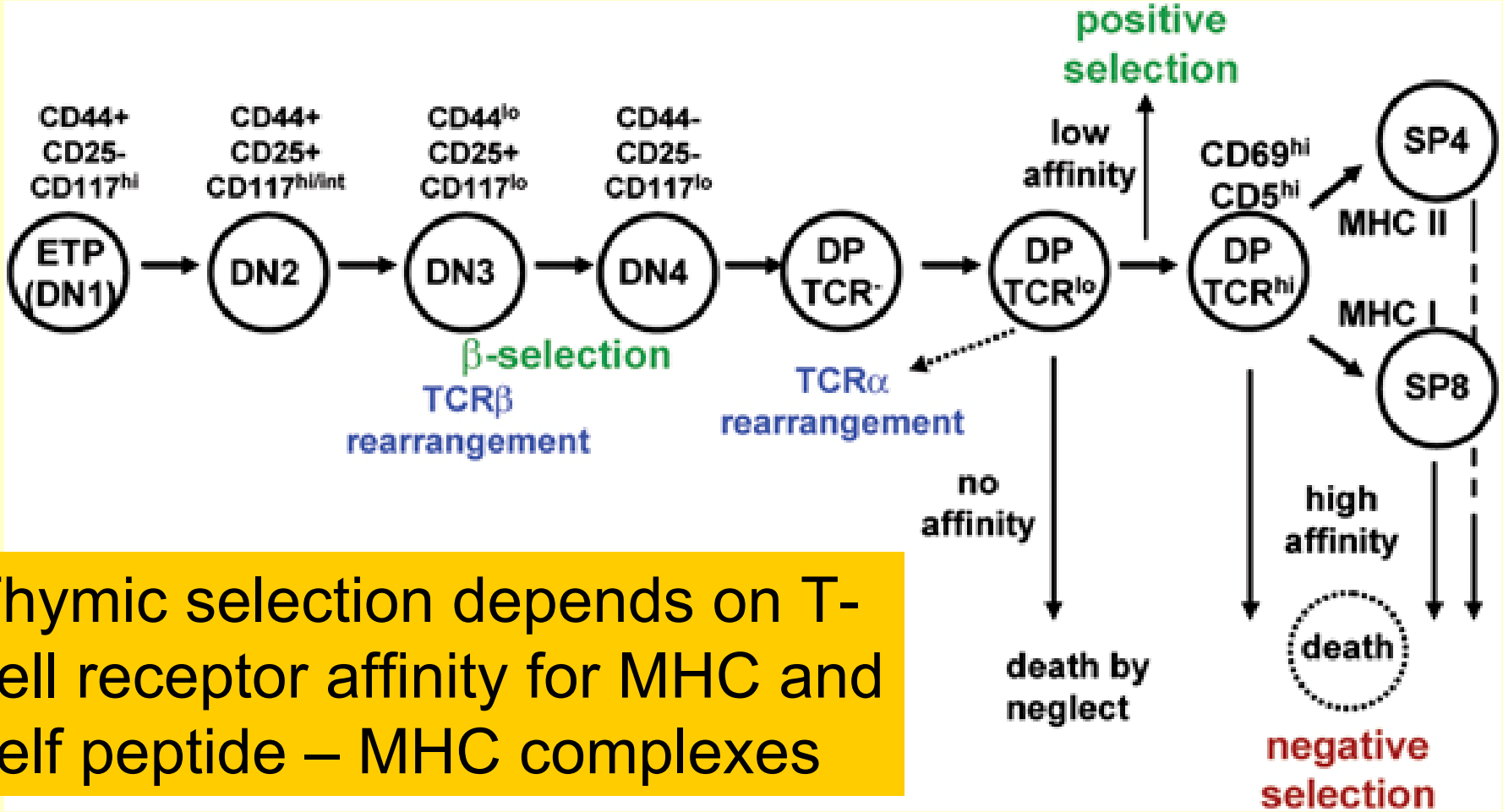
Negative selection: Elimination of self-reactive (“dangerous”) lymphocytes during the process of their maturation



Kamradt, T. et. al. N Engl J Med 2001;344:655-664

Central tolerance
Theory of clonal selection

(Sir Frank Macfarlane Burnet 1957)



Thymic selection depends on T-cell receptor affinity for MHC and self peptide – MHC complexes

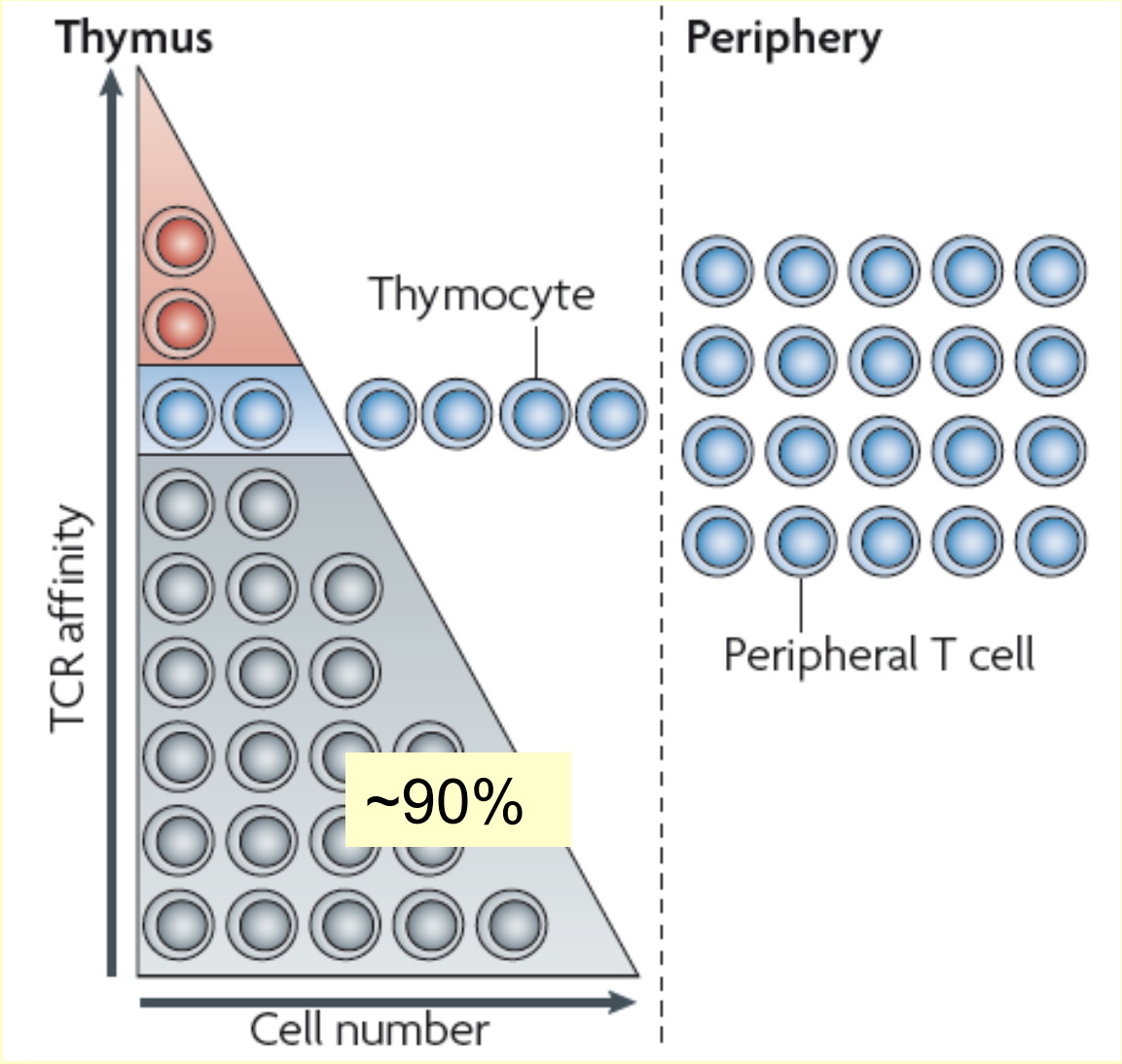
Early thymic progenitors

Double-negative (CD4−CD8−)

Double-positive (CD4+CD8+)

Single-positive (CD4+ or CD8+)

Positive and negative selection



Death by neglect

Positive selecton

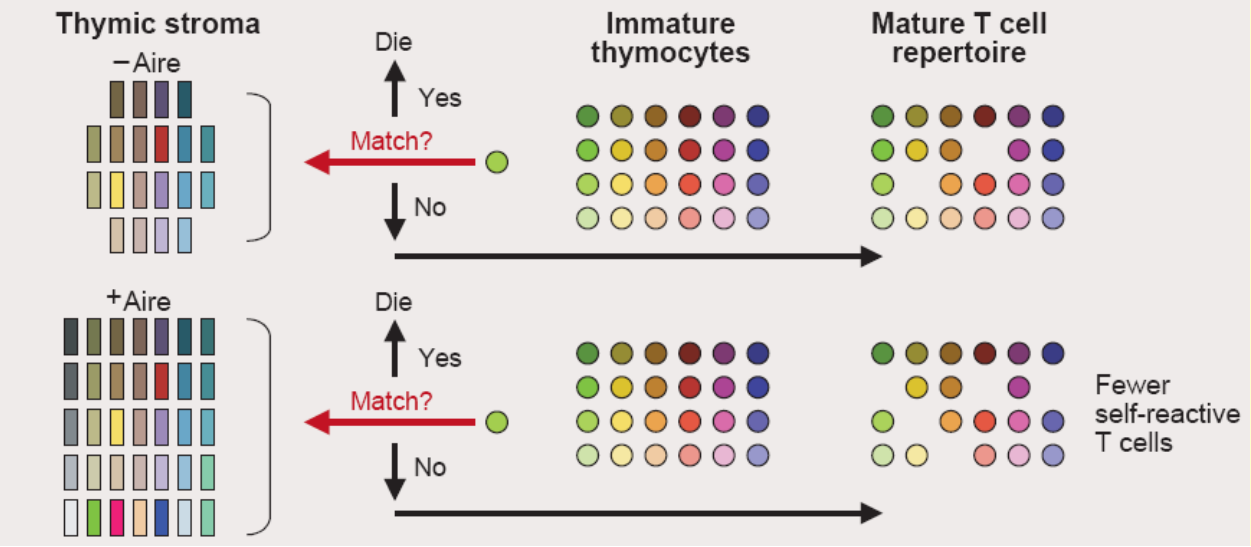
Negative selecton

What happens with negatively selected cells?

- **Clonal deletion** (apoptosis) in self-reactive clones
- **Clonal diversion** of self reactive clones
  - development of Treg cells (CD4+ CD25+ Fox3+)
- Induction of anergy of self reactive clones
- Receptor editing (TCR / BCR)

Are all self protein antigens expressed in the thymus?

- **Autoimmune regulator (AIRE)**
  - transcription factor highly expressed in thymus medulla stromal cells (ch. 21)
  - activation of "tissue-specific" antigen expression in thymus stromal cells!



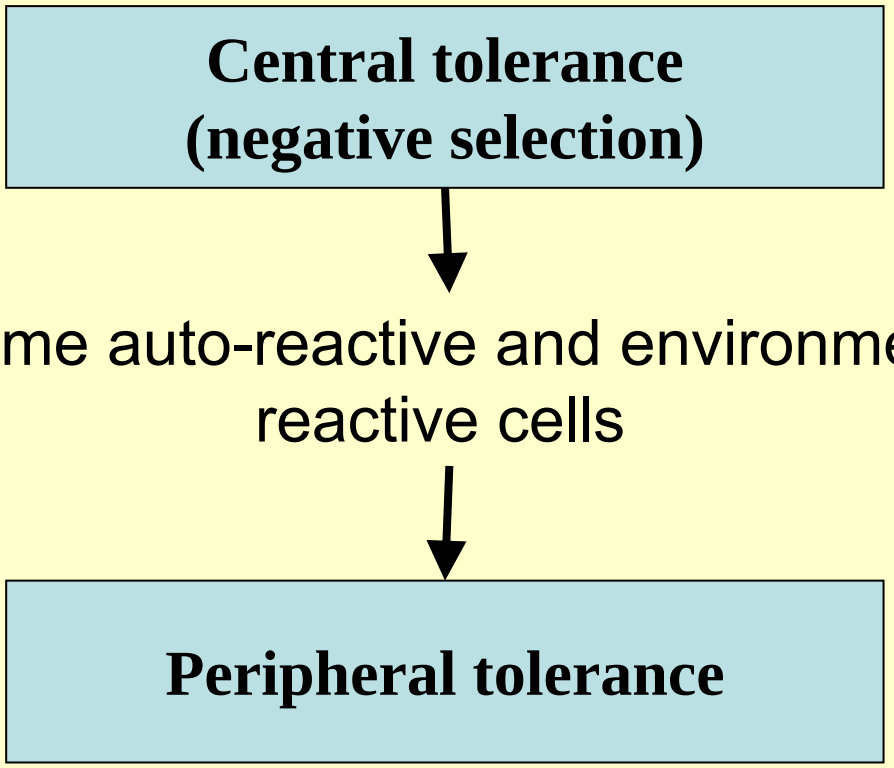
AIRE deficiency

Loss of function mutation

Autoimunne polyendocrine syndrome type I = polyendocrinopathy-candidosis-ectodermal dystrophy (APECED) = Whitaker syndrome

- Failure of central tolerance
  - Autoimmune dysfunction of the
    - parathyroid gland (leading to hypocalcaemia)
    - adrenal gland (Addison's disease: hypoglycemia, hypotension and severe reactions in disease)
    - other tissues hypothyroidism, hypogonadism and infertility, vitiligo (depigmentation of the skin), alopecia (baldness), malabsorption, pernicious anemia, chronic active (autoimmune) hepatitis
- Mild immune deficiency (persistent mucosal and cutaneous infections with candida yeasts)
- Decreased function of the spleen (asplenism)

Mechanism of immune tolerance induction

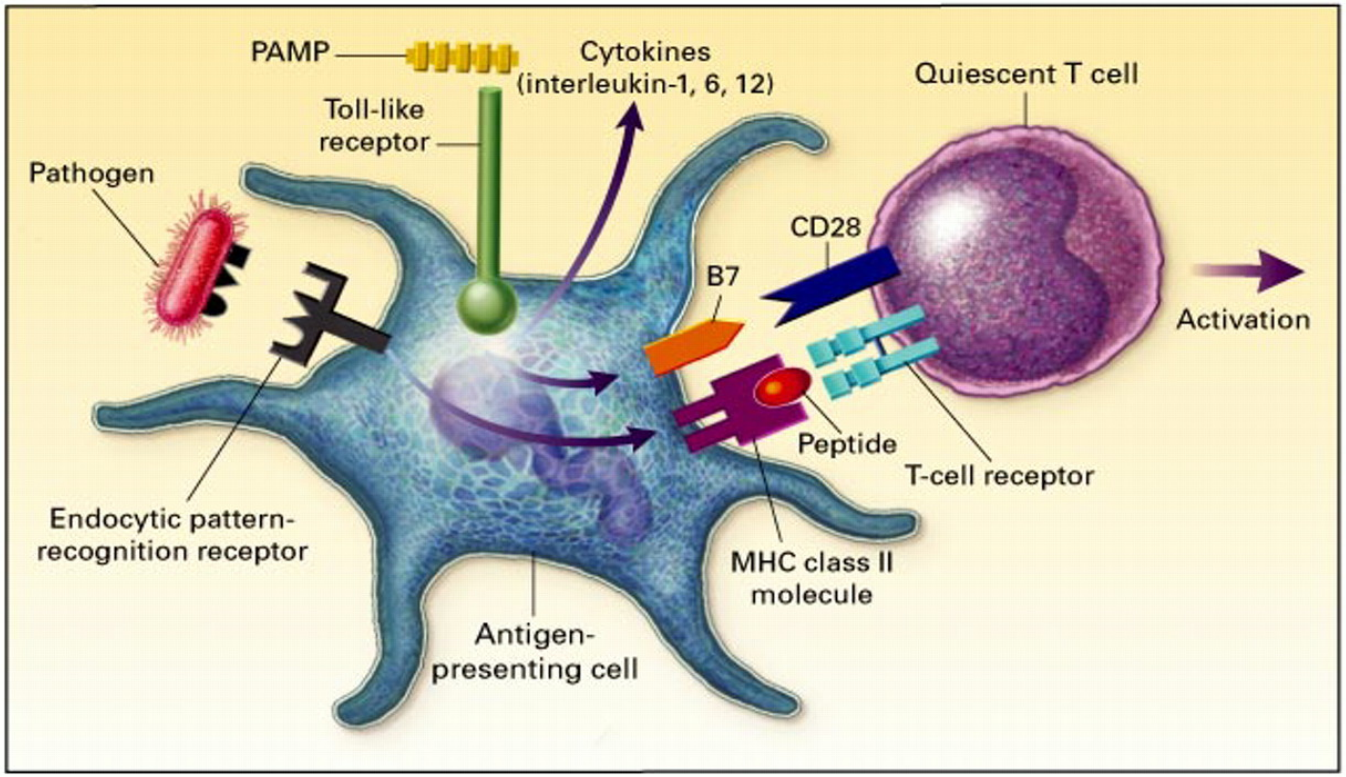


Peripheral tolerance

Elimination of “dangerous” mature lymphocytes

- Mechanism?:
  - Recognition of antigen w/o co-stimulatory signal (2nd signal)

Molecules Involved in the Interplay between Innate and Adaptive Immune Systems



Activation of at least two signaling pathways is necessary to induce immune reaction

Medzhitov, R. et. al. N Engl J Med 2000;343:338-344

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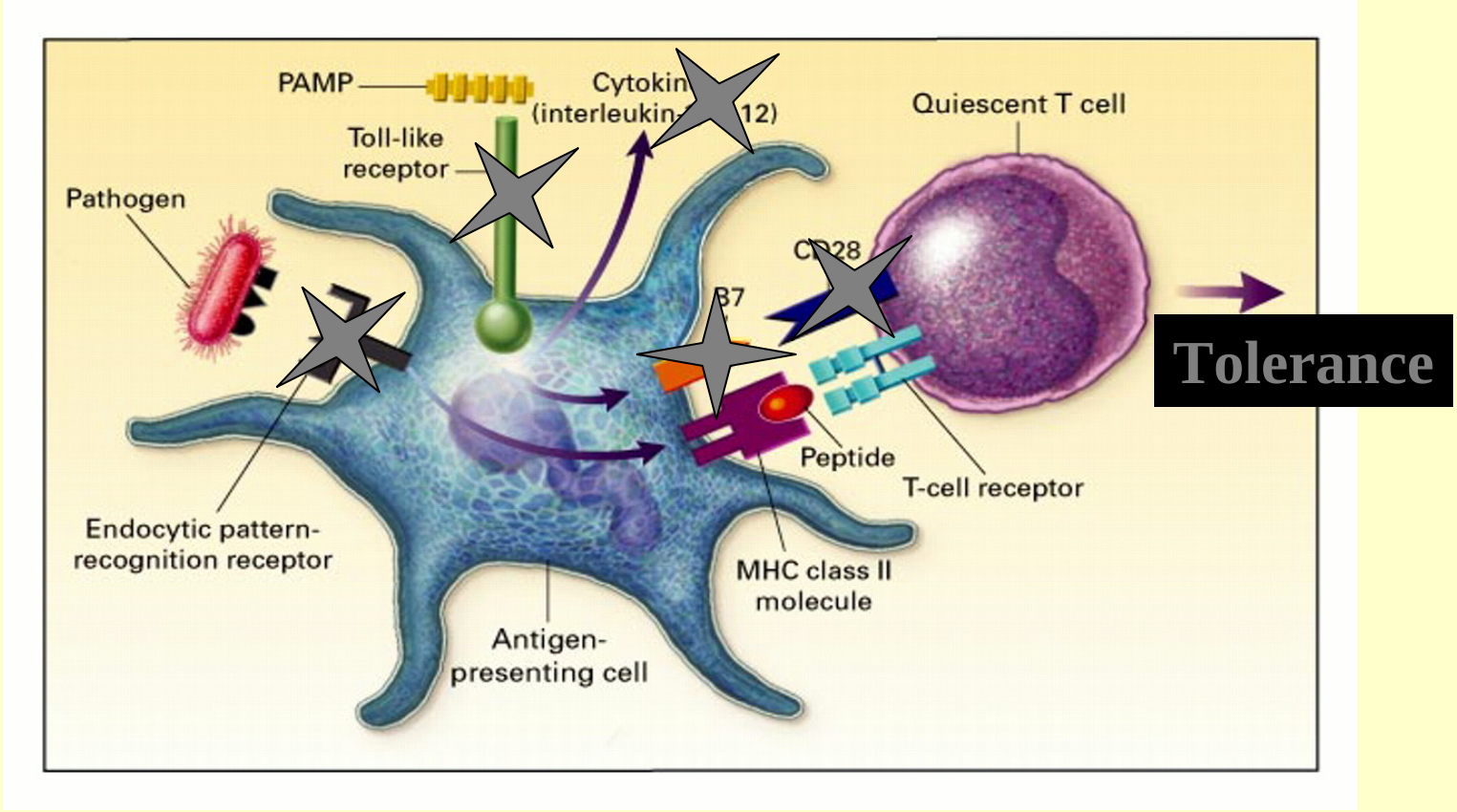

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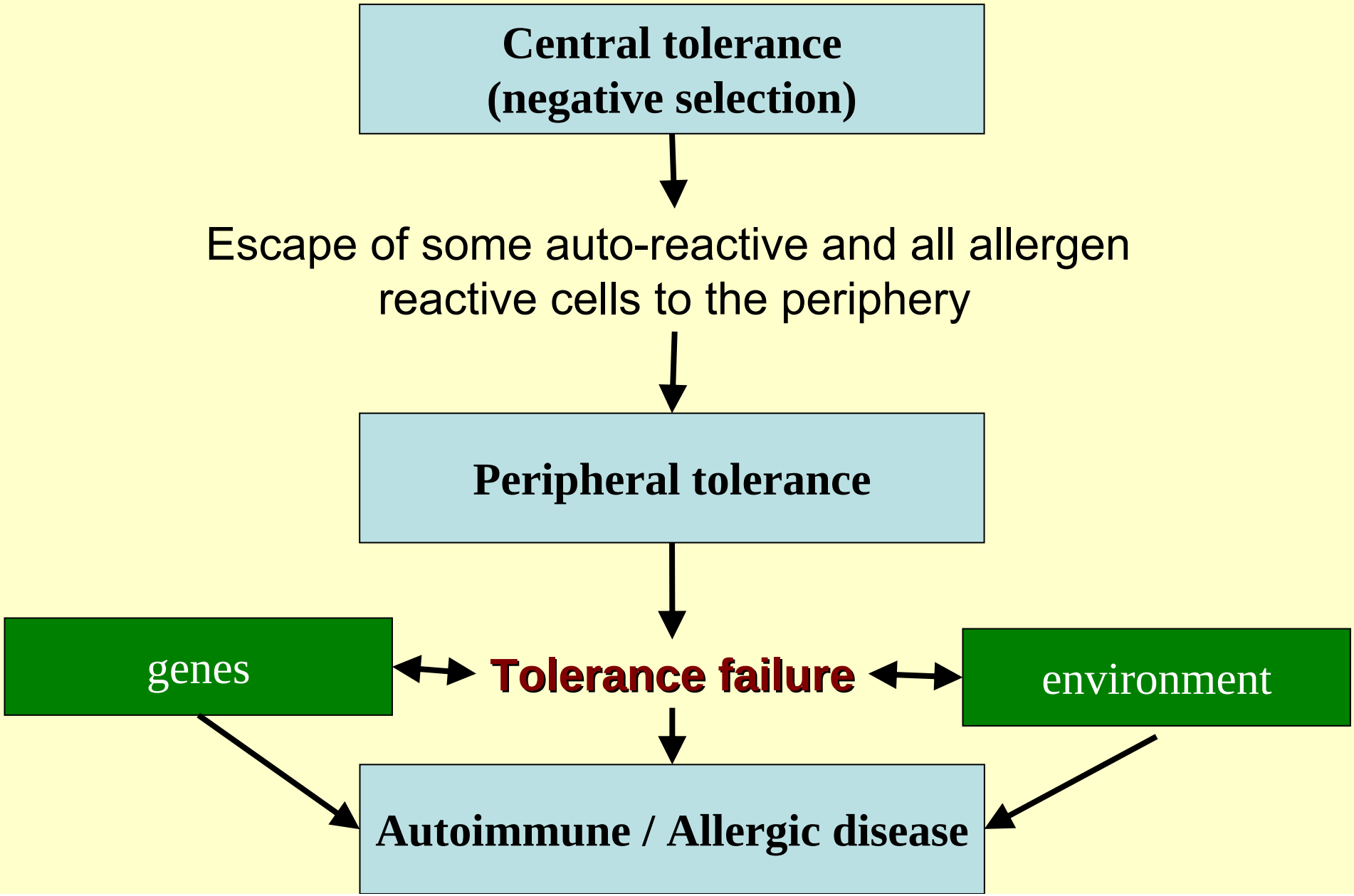
Peripheral tolerance - mechanisms

- **Apoptosis** of reactive lymphocytes
- **Anergy** = functional inactivation
- Induction of antigen-specific regulatory T lymphocytes (**Treg**)
  - production of immunoinhibitory cytokines (IL-10, TGF-β, IL-4)
  - competition for growth factors (IL-2)
  - competition for MHC ligands
  - cytototoxic effect to reactive clones

Mechanism of immune tolerance induction



Failure of immune tolerance autoimmunity (AI) and allergy

- Low levels of immunoreactivity to “self” antigens and common environmental antigens (antibodies, antigen-specific T-cells)
- The central role is played by helper T-cells (**Th:** CD4+) and regulatory T-cells (**Treg:** CD4+ CD25+Fox3+)
- **Homeostasis** Th and Treg

Autoimmune disease development and progression

„Epitope spreading“ hypothesis

Immune reaction is initiated to dominant antigenic epitope

Gradual expansion of pathologic immune reaction to several antigenic epitopes of the primary (auto)antigen and further to other antigens of the same microenvironment.

Leads to clinical manifestation of autoimmune (and allergic) reaction and disease progression

Case report

Medical History

- Male 23 yr.
- 30 minutes after eating pickled sausage with onions felt tension in the epigastrium w/o nausea and w/o vomiting. Tension in the epigastrium persisted till the next day, patient had 3 dark greasy stools. Afternoon his body temperature increased to 38^{o}C.
- Examined by surgeon w/o sign of peritoneal irritation, per rectum normal finding w/o macroscopic hemorrhage
- Referred to the internal medicine department

Medical History

- He has often tension in the epigastrium during the last 2 years after the meal (often dissolved within few hours)
- Frequent bowel movements, stool of mash consistence with mucus
- Weight loss of 4 kg during the last two years
- Has been treated for depressions for two years (Aurorix 1x1 tbl. = reversible inhibitor of monoaminooxidase type A)
- Allergy: Ampicillin and Penicillin

Physical examination

- BP 120/80, P 74/min., BT 36,3 C
- Height 176 cm, Weight 61 kg
- ECG
  - sinus rhythm; HR 74/min; PQ 112 ms; QRS 86 ms
- Differences from normal finding:
  - Pale skin
  - Asthenic physical constitution
  - Tenderness of the whole epigastrium w/o signs of peritoneal irritation

Laboratory

- ESR 6 mm/h
- **Biochemistry:**
  - Na+ 138 (135-145) mmol/l
  - **K+ 3.4 (3.5-5) mmol/l**
  - **Cl- 97 (98-107) mmol/l**

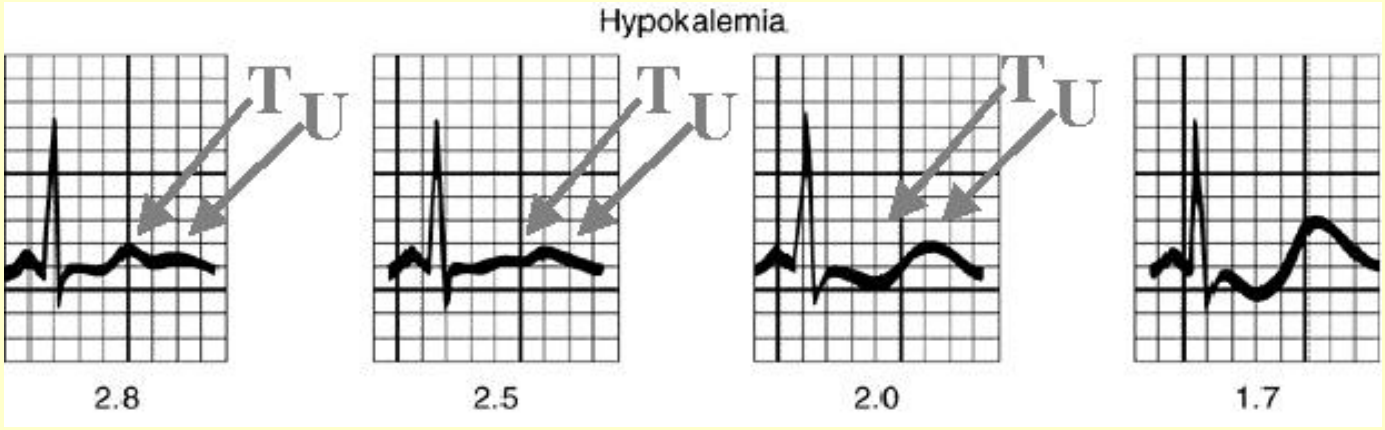
Hypokalemia – cause?

- Insufficient consumption of potassium (in combination with excessive loss)
- GI tract loss of potassium
  - vomiting, diarrhea
- Renal loss of potassium
  - Diabetic ketoacidosis: polyuria and as a partner to the negatively charged ketone and β-hydroxybutyrate
  - Hyperaldosteronism: hypertension and excessive urinary losses of potassium
  - Hereditary defects of ion transporters (Bartter sy., Gitelman sy.)
- Medications: diuretics (hydrochlorothiazide, furosemide); laxatives, amphotericin B)
- Hypomagnesemia (refractory hypokalemia)

Serious hypokalemia ?

- < 3 mmol/L (p.o. supplementation)
  - severe < 2.5 mmol/L; (i.v. supplementation)

- ECG findings associated with hypokalemia?
  - defect in depolarization phase of the heart
  - flattened T waves, apearance of U waves, prolongation of the QT interval, atrial and ventricular arrhythmia



- Other clinical findings?
  - muscular weakness, myalgia, and muscle cramps

What other laboratory tests do you suggest?

Laboratory

- **Urea** 6.6 (2.9-7.1) mmol/l;
- **Creatinine** 96 (50-133) μmol/l
- **Blood glucose** 6.1 (3.3-6.3) mmol/l
- **Liver tests**
  - enzymes: within normal range (ALT; AST; ALP; GMT; CHE)
  - **Bilirubin** 8.2 (5.1-17); μmol/l;
- **Cholesterol** 3.9 (<5.2) mmol/l; **triglycerols** 0.9 mmol/l (<1.8 mmol/l)

Blood count:

- **Leu** **12.7 x 10^9/L (3.4-10 x 10^9/L)**
- Ery 3.45 x 10^{12}/L (4.2-5.6 x 10^{12}/L)
- **Hb** **83 g/L (135-175 g/L)**
- **Hct** **24.1 % (39-49 %)**
  - **MCV** **71 fL (80-100 fL)**
  - **MCH** **25 pg (26-34 pg)**
- Plt 204 x 10^9/L (150-450 x 10^9/L)
- **Coagulation:**
  - Quick INR 1.0
  - APTT 33s (contr. 34s)

What are the causes of microcytic hypochromic anemia?

What is the most frequent cause of microcytic hypochromic anemia?

Differential diagnosis of microcytic anemia

- **Iron deficiency**
- **Inherited defect in globin chain synthesis: the thalassemias**
- **Anemia of chronic inflammation with inadequate iron supply to the erythroid marrow**
- **Myelodysplastic syndromes**

Diagnosis of Microcytic Anemia ?

Laboratory
Evaluation of anemia

- **Serum iron 7.7 μmol/L** (9 to 27 μmol/L)
- Transferrin 3.8 g/L (1.7 - 3.8 g/L)
- **Ferritin 13 μg/L** (30-300 μg/L)
- P-vitamin B12 414 (223-1123) ng/l;
- Folic acid 5.5 (3.1-12.4) μg/l

Differential diagnosis of microcytic anemia

- **Iron deficiency**
  - Low serum iron, low serum ferritin, increased or normal serum transferrin
- **Inherited defect in globin chain synthesis**: the thalassemias
  - normal or increased serum iron levels and transferrin saturation
  - red blood cell distribution width (RDW) index is generally small
- **Anemia of chronic inflammation** with inadequate iron supply to the erythroid marrow (often normocytic and normochromic)
  - low serum iron, normal or increased serum ferritin, decreased serum transferrin (low TIBC)
- **Myelodysplastic syndromes**
  - ocasionally impaired hemoglobin synthesis with mitochondrial dysfunction, resulting in impaired iron incorporation into heme.
  - normal or increased serum ferritin
  - more than an adequate supply to the marrow (sideroblastic anemia)

Laboratory
Evaluation of anemia

- **Serum iron 7.7 μmol/L** (9 to 27 μmol/L)
- Transferrin 3.8 g/L (1.7 - 3.8 g/L)
- **Ferritin 13 μg/L** (30-300 μg/L)
- P-vitamin B12 414 (223-1123) ng/l;
- Folic acid 5.5 (3.1-12.4) μg/l
- **Direct Coombs test positive**

Iron deficiency anemia

with possible hemolytic anemia component

Cause of iron deficiency?

Causes of Iron Deficiency

- **Increased Demand for Iron**
  - **Rapid growth in infancy or adolescence**
  - **Pregnancy**
  - Erythropoietin therapy
- **Increased Iron Loss**
  - **Chronic blood loss**
  - Menses
  - Acute blood loss
  - Blood donation
  - Phlebotomy as treatment for polycythemia vera
- **Decreased Iron Intake or Absorption**
  - **Inadequate diet**
  - Malabsorption from disease (sprue, Crohn's disease)
  - Malabsorption from surgery (postgastrectomy)
  - Acute or chronic inflammation

Cause of patients iron deficiency?

- Gastrointestinal bleeding
- Urogenital bleeding
- Other (inadequate diet or iron resorption)

Gastrointestinal bleeding

- Vomiting of blood (hematemesis)
- Passage of gross blood through the rectum (hematochezia)
- Passage of black tarry stool (melena)
- **Occult chronic bleeding from the GI tract**

Laboratory

- Urine chemistry and sediment:
  - leukocytes +, few bacteria
  - No erythrocytes or hemoglobinuria

- Stool for occult blood
  - positive

Differential diagnosis?

Differential Diagnosis

- GI inflammatory diseases
  - IBD (ulcerous colitic, Crohns disease
  - Peptic ulcer disease
  - Drug related enterocolitis (NSAID, chemotherapy, cocaine)
  - Ischemic enterocolitis

Differential Diagnosis

- GI chronic infectious diseases
  - Bacterial
    - *H. pylori, Salmonella, Shigella, E. Coli, Campylobacter, Yersinia, Clostridium difficile, Gonorrhea, Chlamydia trachomatis*, Mycobacterial infections *(Tuberculosis)*
  - Parazite (*Amebiasis)*
    - Entamoeba histolytica or related species infect about 10% of the world's population
  - Viral
    - *CMV, HSV, HIV*
  - Fungal
    - *Candida, Aspergilus*

Differential Diagnosis

- GI or other abdominal neoplasia
  - Lymphoma, Matastasis
  - Ileal cancer, Carcinoid, Familial polyposis
- Vascular malformations of GI

Laboratory (also done)

- Proteins and acute phase proteins:
  - Total proteins 70 (60-80) g/l
  - Albumin 36 g/l
  - (34-47g/l)
  - Transferrin 3.6 g/l (2.3 –3.8 g/l)
  - Prealbumin 0.3 g/l (0.2-0.36 g/l)
  - CRP 66 μg/l (20-8000 μg/l)
  - α1-antitrypsin 3.4 g/l (0.8-2.1 g/l)
  - β2-microglobulin 2.0 g/l (1.2-2.8 g/l)
  - haptoglobin 2.8 g/l (0.16-1.99 g/l)
- Immunoglobulins
  - IgA 4.64 g/l (0.70-3.85 g/l);
  - IgG 11.3 g/l (6.90-15.7 g/l);
  - IgM 2.8 g/l (0.55-2.30 g/l)

Diagnosis?

Clinical imaging methods

- **Abdominal ultrasound:**
  - Liver of normal size with normal echogenity and structure. Gall-bladder and bile ducts normal finding. Pancreas, kidneys and spleen are normal.
- **Abdominal CT:**
  - Native CT and after the administration of contrast substance. Liver of normal size with normal structure. Bile ducts are normal. Pancreas, of normal size and structure. Kidneys of normal size and structure with nornal urethers. Thickened wall of terminal ileum. Lymphadenopaties not detected.

Clinical examinations

- Gastroscopy:
  - Irritative stomach, normal finding in the area from esophagus to D2 segment of duodenum.
- X-ray of small intestine in double contrast:
  - Two stenotic segments of ileum (15 and 10 cm), Contrast substance filling a communication between ileum and ascendant part of large bowel. Typical finding for Crohn's disease.
- Coloscopy:
  - Thickened wall of large bowel near hepatic flexure - biopsy taken. Otherwise, normal finding in the area from colon transversum to rectum.

CROHN'S DISEASE

Inflammatory bowel disease (IBD)

Incidence: 0.7–9.8 / 100,000 person / year (Europe)

Inflammatory bowel diseases (IBD)

- Heterogeneous group of diseases
- Characterized by: chronic inflammation with periods of exacerbation and remission of GIT
- Chronic unexpected course
- IBD runs in families.
  - The lifetime risk that a first-degree relative will be affected is ~10%
  - If two parents have IBD, each child has a 36% chance of being affected.

MALT

- 400 m^2 (skin 1.8 m^2)
- ~ 80% of all immunocompetent cells
- high production of IgA (S-IgA)

Failure of Gut-associated lymphoid tissue (GALT) function

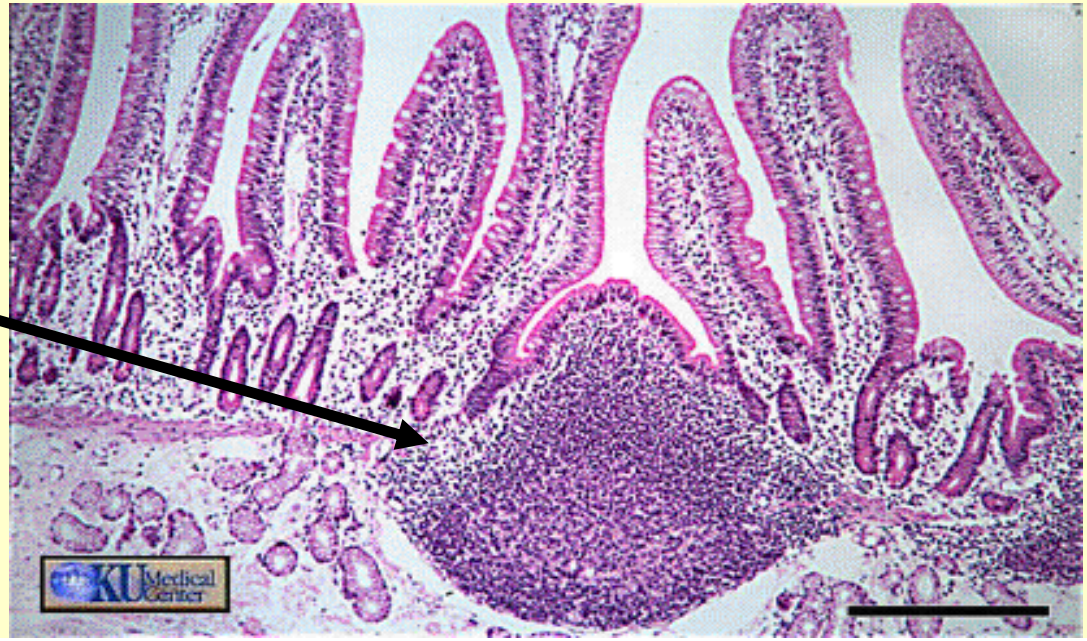
- Normal intestines contain a large number of immune cells in a chronic state of so-called physiologic inflammation

Gut-associated lymphoid tissue (GALT)

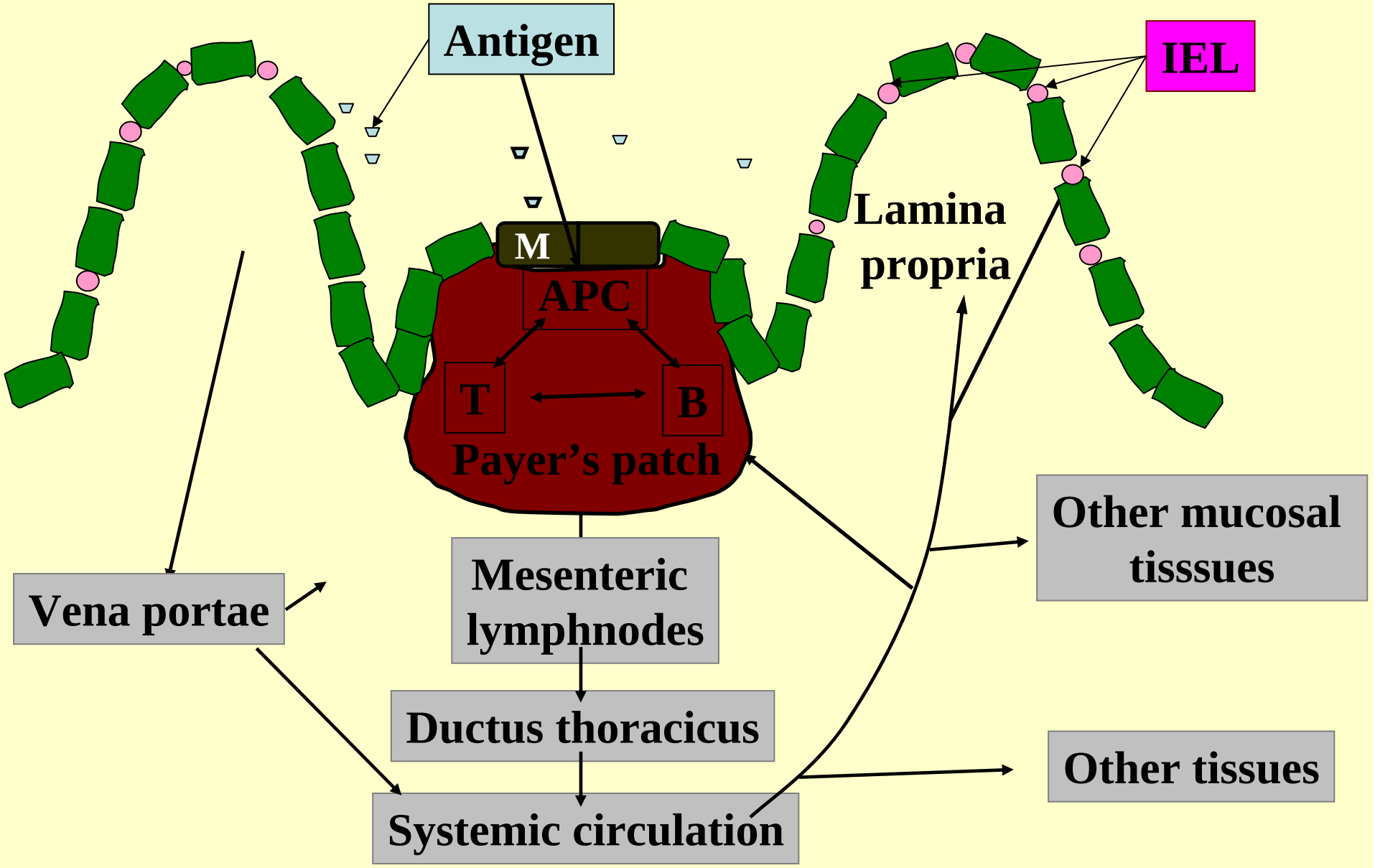
Inductive sites

- antigen processing
- primary stimulation of T a B lymphocytes
  - Payer‘s patches, isolated lymphoid follicles, appendix, lymph nodes

Duodenum – Payer’s patch



The inductive and effector sites of the GALT



Gut-associated lymphoid tissue (GALT)

Effector sites

- Gut lamina propria
  - Lymphocytes with activated phenotype

- Intraepithelial compartment
  - T lymphocytes, most CD8, 10% γ/δ TCR
  - 10-40 IEL/100 enterocytes

Genetic Considerations

- Polygenic disorder that gives rise to multiple clinical subgroups within UC and CD.
- Identified > 100 disease-associated loci on many different chromosomes
- Overlaping pathogenesis between CD and UC
  - 1/3 of genetic risk factors are shared
  - epidemiologic observations of both diseases in the same families
  - similarities in response to therapies
- Many of the genetic risk factors identified are also observed to be associated with risk for other immune-mediated diseases
  - rheumatoid arthritis, psoriasis, ankylosing spondylitis (Bechterew's disease), DM type 1, asthma, SLE

Genetic Loci Associated with CD (and/or UC)

- **Innate Immunity and Autophagy**
  - to respond to and clear bacteria, mycobacteria and viruses
  - Intelectin 1 (Bacterial binding)
  - Autophagy related 16-like 1 (*ATG16L1* – Autophagy)
  - Janus kinase 2 (*JAK2* - IL-6R & IL-23R signaling)
  - Nucleotide-binding oligomerization domain containing 2 (*NOD2* - Bacterial sensing)
- **ER Stress and Metabolic stress**
  - Solute carrier family 22, member 5 (*SLC22A5* / beta-carnitine transporter
  - Anterior gradient 2 (*AGR2* - ER stress)
- **Adaptive Immunity**
  - Interleukin 23 receptor (*IL23R* - Th17 cell stimulation)
  - Interleukin-10 (*IL10* - Treg associated cytokine)
  - Interleukin 12B (IL-12 p40 chain of IL-12/IL-23)
- **Inflammation**
  - Macrophage stimulating 1 (*MST1* - Macrophage activation)
  - Prostaglandin E receptor 4 (PGE2 receptor)
  - Chemokine (C-C motif) receptor 6 (*CCR6* - Dendritic cell migration)

Primary Genetic Disorders Associated with IBD

- Turner's syndrome
  - Loss of part or all of X chromosome
  - UC and colonic CD
- Hermansky-Pudlak
  - Autosomal recessive chromosome 10q23
  - Granulomatous colitis, oculocutaneous albinism, platelet dysfunction, pulmonary fibrosis
- Wiskott-Aldrich syndrome (WAS)
  - X-linked recessive - loss of WAS protein function
  - Colitis, immunodeficiency, severely dysfunctional platelets, and thrombocytopenia
- Glycogen Storage disease
  - Deficiency of the glucose-6-phosphate transport protein type B1
  - Granulomatous colitis, presents in infancy with hypoglycemia, growth failure, hepatomegaly, and neutropenia
- Immune dysregulation polyendocrinopathy, enteropathy X-linked (IPEX)
  - Loss of FoxP3 transcription factor and T regulatory cell function
  - UC-like autoimmune enteropathy, with endocrinopathy (neonatal type 1 diabetes or thyroiditis), dermatitis
- Early onset IBD
  - Deficient IL-10 receptor function
  - Severe, refractory IBD in early life

Etiology and pathogenesis

- Exogenous factors
  - e.g., composition of normal intestinal microbiota
- Endogenous host factors
  - e.g., intestinal epithelial cell barrier function
  - innate and adaptive immune function
- Dysregulated mucosal immune function that is further modified by specific environmental factors
  - e.g., smoking, enteropathogens
- Appropriate response to an unidentified infectious agent???
- Inappropriate immune response to the endogenous commensal microbiota within the intestines, with or without some component of autoimmunity.

Mechanisms involved in the induction of oral tolerance

- deletion of antigen-reactive T cells
- anergy of antigen-reactive T cells
- induction of CD4+ T regulatory cells that suppress gut inflammation (secretion of anti-inflammatory cytokines: IL10, TGF-beta)

Defective Immune Regulation in IBD

- Gene knockout (–/–) or transgenic (Tg) mouse models of IBD
  - deleting specific cytokines (e.g., IL-2, IL-10, TGF-) or their receptors
  - deleting molecules associated with T cell antigen recognition (e.g., T cell antigen receptors)
  - interfering with intestinal epithelial cell barrier function and with regulation of responses to commensal bacteria (e.g., XBP1, N-cadherin, mucus glycoprotein or NF-kB)
  - Intestinal inflammation in these animal models requires the presence of the commensal microbiota.
  - Human IBD inappropriate responses of the genetically susceptible host to the commensal bacteria.
- Inappropriate innate immune sensing and reactivity to commensal bacteria
- Inadequate regulatory pathways that lead to activated CD4+ helper T cells in the lamina propria that secrete excessive quantities of inflammatory cytokines relative to anti-inflammatory cytokines.
- Activation of other inflammatory cells (macrophages and B cells)
- Recruitment of lymphocytes, inflammatory leukocytes, and mononuclear cells from the bloodstream into the gut through interactions between homing receptors on leukocytes and addressins on vascular endothelium.

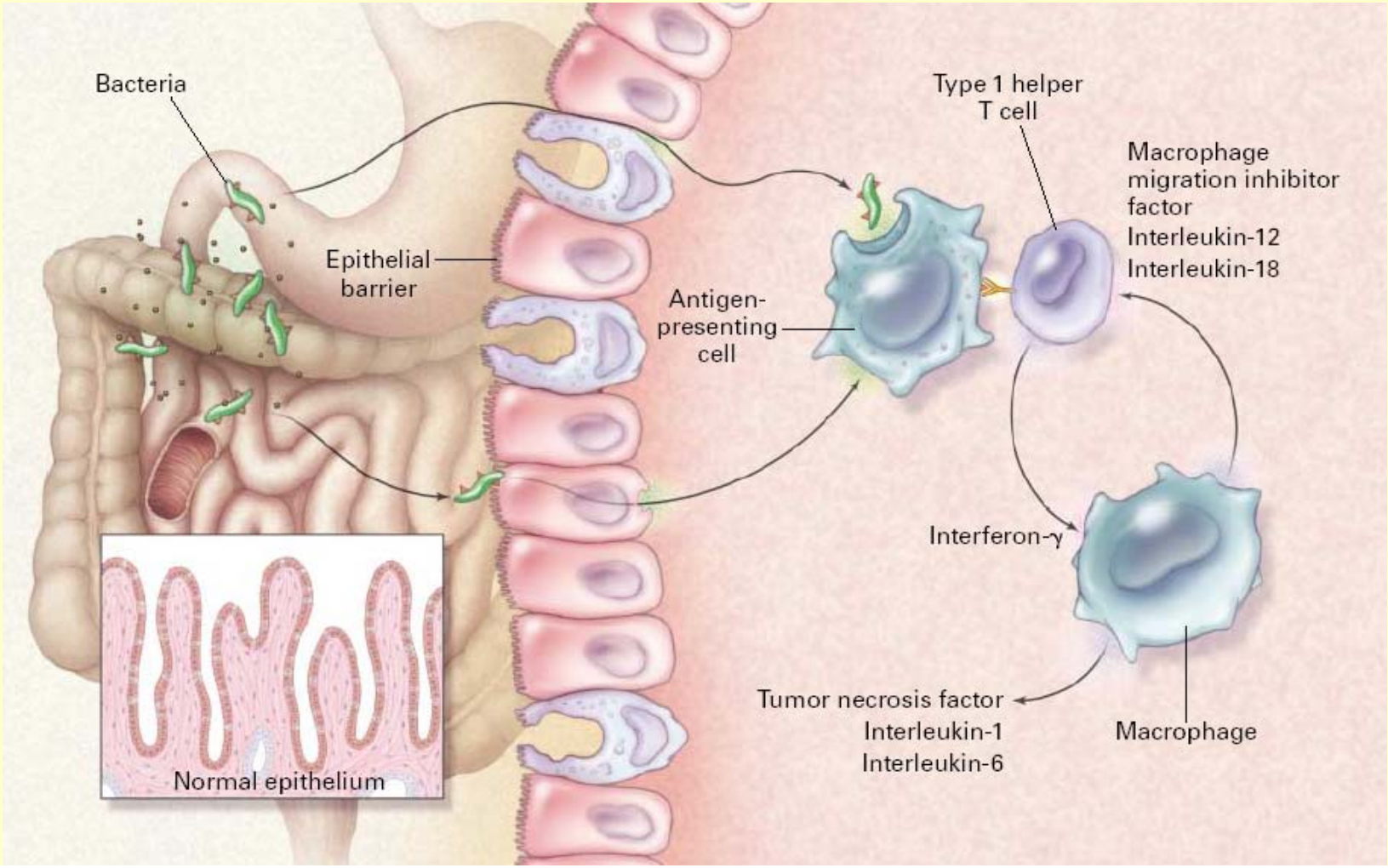
Immune mechanisms

- IBD patients and experimental animal models have
  - Increased T lymphocyte reactivity and antibodies to gut comensal bacteria antigens
- No evidence for association with known infections
- Experimental animal models of IBD do not develop disease when grown in microbial free environment (e.g. IL-10-/-)

Breakdown of immune tolerance to comensal bacteria?

Pathogenesis of Inflammatory Bowel Disease

- Breakdown of intestinal epithelial barrier function
- Failure of immune regulatory mechanisms



Types of CD4+ T helper (Th) cells associated with colitis in animal models

- TH1 cells (secrete IFN – gamma)
  - initiated by IL-12, a key cytokine in the pathogenesis of experimental models of mucosal inflammation
  - transmural granulomatous inflammation that resembles CD
- TH2 cells (secrete IL-4, IL-5, IL-13) and related natural killer T cells
  - initiated by IL-4 (with IL-6 and TGF-beta)
  - induce superficial mucosal inflammation resembling UC
- TH17 cells (secrete IL-17, IL-21)
  - initiated by IL-23 (with IL-6 and TGF-beta)
  - responsible for neutrophilic recruitment

THE INFLAMMATORY CASCADE IN IBD

- Inflammatory response is perpetuated as a consequence of T cell activation
- Inflammatory cytokines
  - e.g. IL-1, IL-6, and TNF
    - promote fibrogenesis, collagen production, activation of tissue metalloproteinases, and the production of other inflammatory mediators
  - Local activation of hemostasis
    - e.g. increased production of von Willebrand's factor

Crohn‘s disease

regional enterocolitis; granulomatose colitis
(1932, JAMA: Crohn, Ginzberg, Oppenheimer)

- **Segmental transmural** inflammation of GIT
- **Spreads discontinuously** – usually in submucosa
- Involvement of **serosa = adhesions** and development of pathologic communications (fistulas)
- Involvement of **mucosa = erosions and ulcerations** and bleeding
- Usually in the **ileum**, but can affect **any part of GIT** including mucosa of oral cavity - The site of disease influences the clinical manifestations.

Crohn's disease pattern

- Presents as acute or chronic bowel inflammation
- Inflammatory process evolves towards
  - a fibrostenotic obstructing pattern
  - penetrating fistulous pattern

Ulcerative colitis

- diffuse inflammation of large bowel
- in more then 95% of cases starts at rectum and spreads continuously proximally
- Superficial mucosal inflammation can affect also submucosa but usually does not reach deeper layers (serosa)

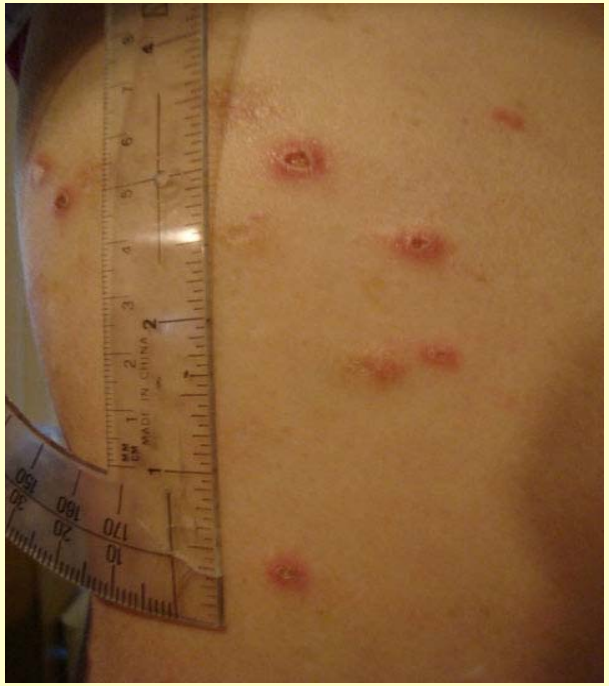
Complications of CD

- Serosal adhesions and fistula formation
- Intestinal obstruction (40% of patients)
- Intraabdominal and pelvic abscesses (10–30% of patients)
- Chronic hemorrhage
- Perforation (1–2% of patients)
- Malabsorption
- Toxic megacolon (may perforate)
- Massive hemorrhage
- Severe perianal disease

Extraintestinal manifestation of CD

- Skin inflammations:
  - Erythema nodosum (15% CD)

Erythema nodosum



Extraintestinal manifestation of CD

- Skin inflammations:
  - Erythema nodosum (15% CD)
- Rheumatologic
  - periferal arthritis (15-20%)
  - ankylosing spondylitis (10%)
- Eyes inflammations (1-10%)
  - episcleritis, keratoconjuctivitis, iritis
- Liver inflammations
  - sclerosing necrotising cholangiitis (1-5%)
- Urologic
  - Tubulointersticial nephritis
  - Nefrolithiasis (10-20%)
- Other (thrombosis, hemolysis, embolisation)

DIFFERENTIAL DIAGNOSIS OF UC AND CD

- Indeterminate colitis
  - IBD where distinguishing between UC and CD is impossible (10 to 15% of cases)

Serologic Markers

- To differentiate between CD and UC
  - Perinuclear antineutrophil cytoplasmic antibodies (pANCAs) are associated with UC
    - positivity in 60 to 70% of UC patients, 5 to 10% of CD patients, and 2 to 3% of the general population
  - Anti-Saccharomyces cerevisiae antibodies (ASCAs)
    - recognize mannose in the cell wall mannan of S. cerevisiae
    - 60 to 70% of CD patients, 10 to 15% of UC patients, and up to 5% of non-IBD controls
  - pANCA+ / ASCA- 57% sensitivity and 97% specificity for UC
  - pANCA- / ASCA+ 49% sensitivity and 97% specificity for CD

Experimental models of IBD

- Spontaneous IBD like disease:
  - SAMP1/Yit mouse (ileitis, CD model)
  - C3H/HejBir mouse (colitis)
  - cotton top Tamarin
- Induced
  - Administration of exogenous substances
    - Enema – TNBS, Oxazolon, Acetic acid, Immune-complex-formalin
    - p.o. – Indomethacin, DSS, carageenan
    - s.c. – CyclosporinA
    - into the intestine – peptidoglycan polysachride
  - Gene mutations (k.o./transgenic)
    - Cytokine genes: IL-2-/-, IL-Ra-/-, IL-10-/-, TGFb-/-, TNFDARE-/-, CRFB4-/-, IL-7 transgenní, Stat-4 transgenní
    - Function of T cells: TCRa-/-, TCRb-/-, Gai2-/-, MHC II-/-, HLA-B27 tg rat
    - IEC barrier function: mdr1a-/-, N-Cadherin dominant negativ, trefoil faktor-/-
- Cell transfer to the immunocompromised host
  - CD4+RBhi into SCID or Rag-/-
  - BM into Tge26 mouse

Gluten-sensitive enteropathy

Gluten-sensitive enteropathy

coeliakie, gluten enteropathy

- Described in 1888 (Gee)
- Association with gluten found in 1947 (Dicke)
  - –group of proteins present in wheat, ray, barley, (oats) (dominant allergen α-Gliadin)
- Estimated prevalence in North America and Europe 1:100 to 1:300

Gluten-sensitive enteropathy – Etiology

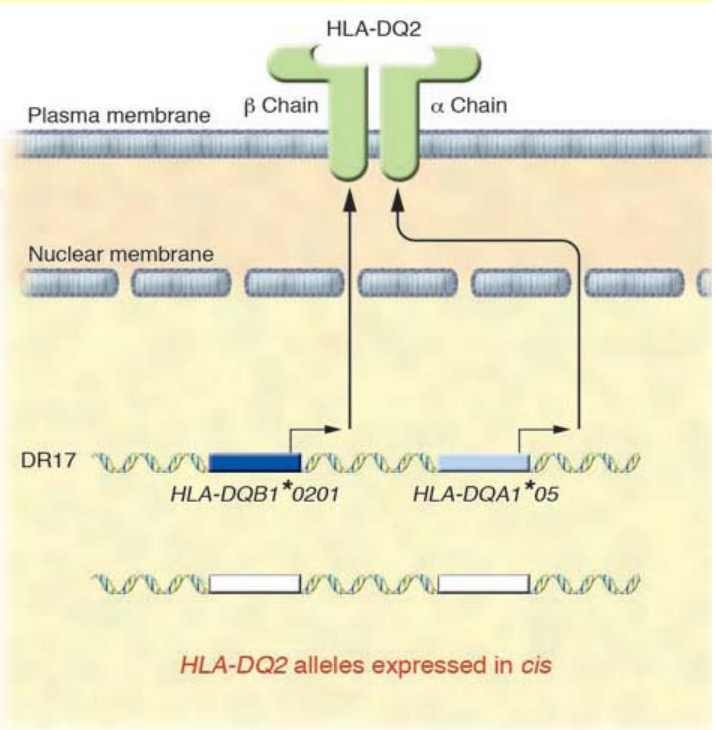
Genetic factors

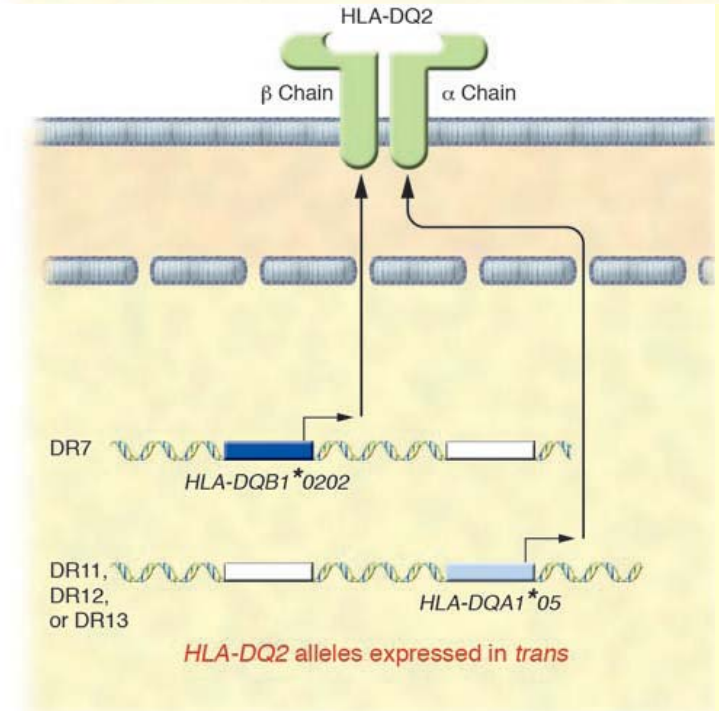
Environmental factors

Immunologic factors

Genetic factors

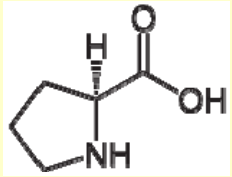
- In first-degree relatives of patients 10% incidence
- More than 95% of patients have HLA DQ2 or DQ8
  - only 40% of general population have HLA DQ2 or DQ8

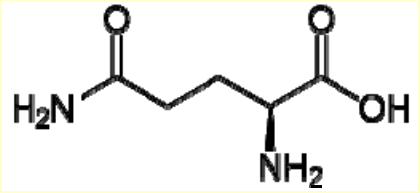




Environmental factors

- “Gluten” (Prolamin) proteins
  - Proteins rich in proline (prol-) and glutamine (-amin)



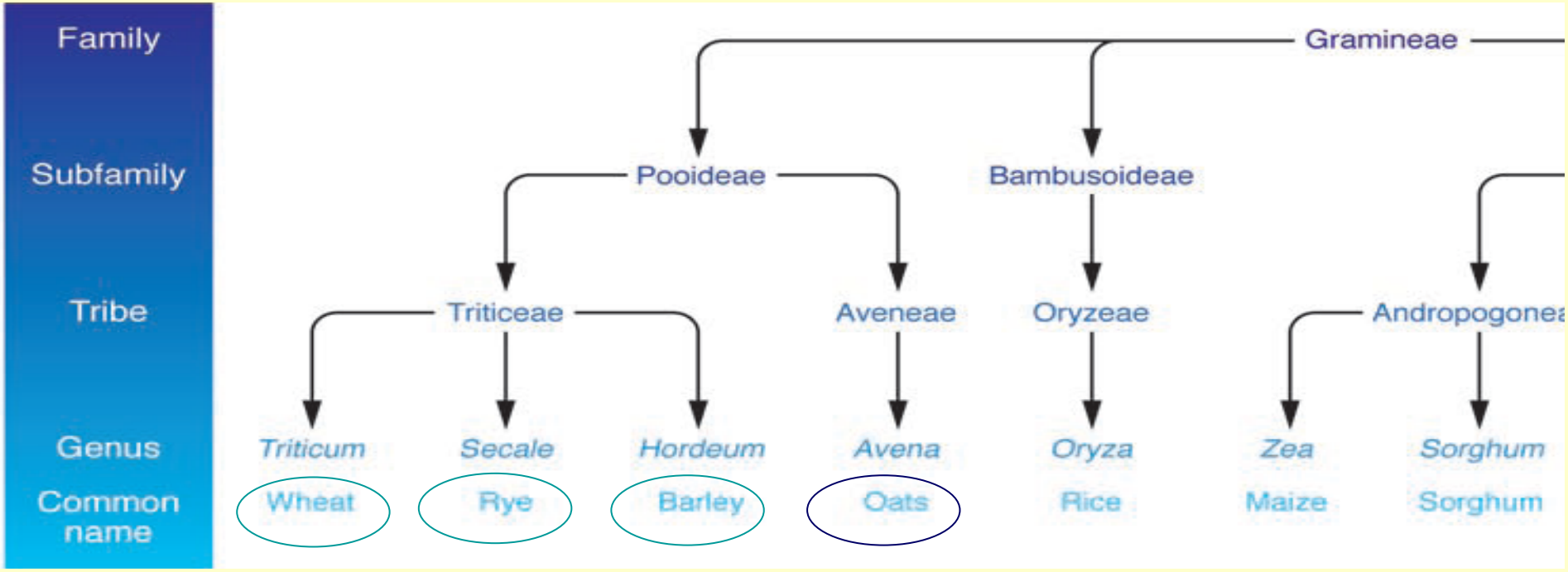


  - Water insoluble proteins
  - Resistant to complete proteolytic digestion by gastric, pancreatic, and brush border enzymes in the human intestine
  - Peptide fragments with high proline and glutamine include “disease-activating peptides”

“Gluten” (Prolamin) proteins

- Wheat, rye, and barley have a common ancestral origin in the grass family
- Oats are thought to activate coeliakie only rarely

Taxonomy of some dietary grains



Gliadin Secalin Hordein Avenin

Gluten-sensitive enteropathy – Pathogenesis

- “Gluten” “disease-activating peptides” rich in proline and glutamine
  - gain access to APCs in the subepithelial region of the small intestine
  - Native forms of “disease-activating peptides” are not able to bind “disease related” HLA-DQ2 (or HLA-DQ8)
    - favors the binding of peptides with negatively charged residues (native “gluten” rather positively charged residues)
  - Tissue Transglutaminase (TGase)
    - modification of “gluten” peptides

Tissue Transglutaminase (TGase)

- Function:
  - tissue repair
    - cross-links proteins by forming isopeptide bonds between glutamine and lysine residues (lysine is not in “gluten” peptides)
- Celiakie:
  - high avidity of TGase for glutamine- and proline-rich “gluten” peptides
  - In the absence of lysine residues and in low pH can deamidate glutamine to negatively charged glutamic acid
    - increase binding affinity for HLA-DQ2 (or HLA-DQ8) molecules

Pathophysiology

"Gluten disease-activating peptides"

HLA-DQ2 and HLA-DQ8

Tissue Transglutaminase (tTGase)

Gluten-sensitive enteropathy – Pathogenesis

- HLA-DQ2 (or –DQ8) on intestinal subepithelial APCs present modified "gluten" peptides to **lymphocytes** (T - helper)
- Activation of pathogenic CD4+ T cells
  - ?
  - "gluten"-specific CD4+ T cells become committed to Th1 cytokine production

Gluten-sensitive enteropathy – Pathogenesis

- Release of cytokines (IFN-γ) and activation/release of enzymes (MMPs) that can damage the mucosa
  - Perpetuate the ongoing immune response
  - Alter mucosal functions including intestinal permeability
  - Results in a loss of villous structure and crypt hypertrophy

Immunologic factors

- “Gluten” – specific cellular and humoral response
  - IgA (IgG) antiendomysial antibodies
    - 90 to 95% sensitivity and 90 to 95% specificit
    - Specific for tissue transglutaminase
  - IgA anti-tissue transglutaminase (tTG) antibodies
  - -specific T cells
    - May mediate tissue injury
- Infiltration of gut mucosa with immune competent cells
- Treatment with prednisolone for 4 weeks in patient who continues to eat gluten will induce a remission

Gluten-sensitive enteropathy - Pathology

- Infiltrative lesions:
  - normal villi with >IEL infiltration (asymtomatic relatives and pts. with dermatitis herpetiformis)
- Hyperplastic lesions:
  - infiltration of villi and crypts with >IEL
- Destructive lesions:
  - atrophy of villi of small intestine
  - defect in enterocyte differentiation
  - inflammatory infiltration of intestinal mucosa

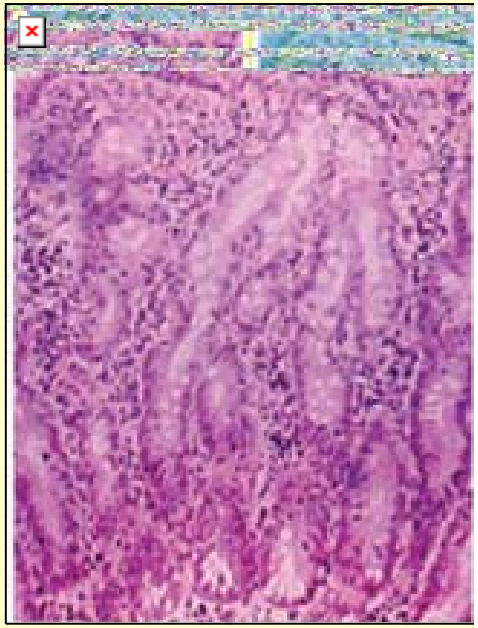
Gluten-sensitive enteropathy - pathology

Normal biopsy

Normal H-E stain

Coeliakie biopsy

Coeliakie H-E stain



Villous atrophy and malabsorption

- Disruption of the structure and function of the small bowel's mucous lining
  - Malabsorption of nutrients, minerals and fat soluble vitamins (A, D, E, K)
  - Lactose intolerance (secondary)
    - present due to the decreased bowel surface and reduced production of lactase by dammaged epithelial cells
    - Typically resolves once the condition is treated

Thank you !